National Research Council Canada

Conseil national de recherches Canada

NRC · CNRC

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

http://www.nrc.ca/cisti/journals

ISSN 0706-652X / CJFSDX 56(10) 1711–1980 (1999)

The *Canadian Journal of Fisheries and Aquatic Sciences*, previously published as *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, and *Journal of the Fisheries Research Board of Canada* 1938–1979, is issued monthly to form annual volumes of 12 numbers plus supplements.

Editorial policy: The Journal publishes original research articles, perspectives (syntheses, critiques, re-evaluations), discussions (comment and reply), and rapid communications. Manuscripts may concern cells, organisms, populations, communities, ecosystems, or processes that affect aquatic systems. They may cover a range of disciplines including biology and ecology of marine and freshwater organisms, limnology, oceanography, physiology, toxicology, genetics, economics, disease, and management. Preference will be given to manuscripts that emphasize understanding of observed phenomena and interpretation of experimental results. The Journal encourages papers that lead from clearly stated purpose or rationale and from testable hypotheses, concepts, or questions to identifiable conclusions or syntheses. Such papers may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. The Journal discourages papers that are essentially descriptive, except in emerging disciplines; have only site-specific or local applications (a certain year, place, taxon, chemical compound, etc.); only confirm previously established principles; or apply standard techniques without breaking new methodological ground.

Manuscripts for publication should be submitted to the Editors, Canadian Journal of Fisheries and Aquatic Sciences, Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada (519-824-4120 ext. 2646; fax: 519-767-9798; e-mail: cjfas@uoguelph.ca).

Submissions in either English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. NRC accepts no responsibility for statements or opinions expressed by a contributor. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the *Journal*'s Editors or NRC. The use of proprietary names does not imply endorsement of the product or company.

Correspondence concerning accepted manuscripts and corrected proofs should be sent to NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

Instructions to Authors are published once a year in the first issue of each volume. Copies are available from NRC Research Press or from our Web site. The **Publication Policy** of NRC Research Press is also available at our Web site:
http://www.nrc.ca/cisti/journals/rj.html

Microfilm: Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, U.S.A.

U.S. periodical postage paid at Champlain, N.Y., and at additional mailing offices. Attention U.S. POSTMASTER: Send address changes to **Canadian Journal of Fisheries and Aquatic Sciences**, c/o USACAN Media Distribution Center, P.O. Box 2888, Plattsburg, NY 12901, U.S.A.

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, et *Journal of the Fisheries Research Board of Canada* 1938–1979. Le *Journal* paraît tous les mois, formant ainsi un volume de 12 numéros, et offre en plus jusqu'à deux suppléments par année.

Politique éditoriale : On publie, dans le Journal, des articles rendant compte de recherche originale, des perspectives (synthèses, critiques et réévaluations), des discussions (commentaires et réponses) et des communications rapides. Les documents peuvent porter sur les cellules, organismes, populations, communautés, écosystèmes ou processus qui affectent les systèmes aquatiques. Ils peuvent relever de divers domaines y compris la biologie et l'écologie des organismes marins et d'eau douce, la limnologie, l'océanographie, la physiologie, la toxicologie, la génétique, l'économie, la pathologie et la gestion. On accorde la préséance aux articles qui contribuent à la compréhension de phénomènes observés et à l'interprétation de résultats expérimentaux. On invite les auteurs à soumettre des articles ayant des objectifs précis ou relevant d'hypothèses, de concepts ou de questions vérifiables et se terminant par de solides conclusions ou d'utiles synthèses. Ces articles enrichissent, changent, mettent en question ou réorientent les connaissances acquises sur divers aspects des sciences aquatiques. On décourage toutefois la soumission d'articles simplement descriptifs (à moins qu'il ne s'agisse de nouvelles disciplines), d'application très limitée (applicables à une seule année, une seule région ou un seul taxon, portant sur un unique composé chimique, etc.); d'articles qui ne font que reprendre les principes énoncés dans des articles précédents; et d'articles rendant compte de l'application de normes sans gain de méthodologie.

Les auteurs voudront adresser leurs **manuscrits** aux directeurs scientifiques, Journal canadien des sciences halieutiques et aquatiques, Département de Zoologie, University of Guelph, Guelph, ON N1G 2W1, Canada (519-824-4120 poste. 2646; télécopieur : 519-767-9798; adresse électronique : cjfas@uoguelph.ca).

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à.-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le CNRC ne se tient en aucune façon responsable des opinions exprimées par un collaborateur ou des déclarations qui pourrait faire ce dernier. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par les directeurs du *Journal* ou du CNRC. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

La correspondance ayant trait aux manuscrits acceptés et les **épreuves corrigés** doivent être envoyées aux Presses scientifiques du CNRC, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Recommandations aux auteurs sont publiés une fois par année dans le premier numéro de chaque volume. Des copies, ainsi que la **Politique de publication**, sont disponibles auprès des Presses scientifiques du CNRC ou au site Web :
http://www.nrc.ca/cisti/journals/rj.html

Microfilms : On peut acheter sur microfilms de 16 ou 35 mm tous les numéros depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, É.-U.

This journal is indexed or abstracted in / Ce journal est signalé ou résumé dans
ABSEARCH, Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Biological & Agricultural Index, CAB Abstracts, Chemical Abstracts, Current Contents, Elsevier BIOBASE, Environmental Abstracts, Environmental Periodicals Bibliography, GEOBASE, GeoRef, Life Sciences Collection, Meteorological and Geoastrophysical Abstracts, Oceanic Abstracts, PASCAL Database, and Science Citation Index.

Volume 56, Number 10, October 1999

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 56, numéro 10, octobre 1999

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

Editors / directeurs scientifiques John C. Roff and Moira M. Ferguson

Assistant to the Editors / adjointe aux directeurs scientifiques **Holly Foster**

Associate Editors / directeurs scientifique associés

D. Boisclair, *Université de Montréal*
U. Borgmann, *Environment Canada, Burlington*
S.E. Campana, *DFO/MPO, Dartmouth*
D.G. Dixon, *University of Waterloo*
A.E. Dizon, *National Marine Fisheries Service, La Jolla, Calif.*
J.J. Dodson, *Université Laval*
J.A. Downing, *Iowa State University*
K.T. Frank, *DFO/MPO, Dartmouth*
D.F. Houlihan, *University of Aberdeen, Aberdeen, Scotland*
F.A. Huntingford, *University of Glasgow*
J.A. Hutchings, *Dalhousie University*
G.S. Jamieson, *DFO/MPO, Nanaimo*
B. Jonsson, *Norwegian Institute for Nature Research, Oslo, Norway*
L. Legendre, *Université Laval*
D.M. Mason, *Purdue University, West Lafayette, Ind.*
A. Mazumder, *University of Victoria*
D.L. Parrish, *Vermont Cooperative Fish and Wildlife Research Unit (USGS/BRD), University of Vermont*
P. Pepin, *DFO/MPO, St. John's*
R.M. Peterman, *Simon Fraser University*
E.E. Prepas, *University of Alberta*
T.M. Powell, *University of California, Berkeley, Calif.*
T.J. Quinn II, *University of Alaska–Fairbanks, Juneau, Alaska*
T.P. Quinn, *University of Washington*
R.D. Robarts, *Environment Canada, Saskatoon*
B. Sainte-Marie, *DFO/MPO, Mont-Joli*
P.F. Sale, *University of Windsor*
D.W. Schindler, *University of Alberta*
S.J. Smith, *DFO/MPO, Dartmouth*
W.G. Sprules, *University of Toronto*
R.J. Thompson, *Memorial University of Newfoundland*
C.J. Walters, *University of British Columbia*
R.D. Ward, *CSIRO Division of Fisheries, Australia*
C.M. Wood, *McMaster University*
B. de Young, *Memorial University of Newfoundland*

NRC Research Press / Presses scientifiques du CNRC

Editor-in-chief / directeur général **Bruce P. Dancik**

Assistant Editor-in-chief / directeur général adjoint **Iain E.P. Taylor**

Journals Program / Programme des revues

(Fax / télécopieur : 613-952-7656; e-mail / adresse électronique : research.journals@nrc.ca;
URL: http://www.nrc.ca/cisti/journals/)

Director / directrice **Aldyth Holmes**

Business Manager / gestionnaire des affaires **Paul McClymont**

Managing Editor / rédactrice administrative **Cecily M. Pearson (613-993-9099)**

Publication Officer / agente de publication **Jennifer McColl**

Advertising / annonces publicitaires **Judy Heyman (613-993-0151)**

Reprints / tirés à part **Roxanne Landriault (613-990-2254)**

Subscriptions / abonnements **Helen Goulet (613-993-9084)**

© National Research Council of Canada 1999. World rights reserved.
No part of this publication may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the publisher, except as stated below.

The National Research Council of Canada grants **permission*** to individuals to make single copies of articles for personal use. Any person may reproduce short excerpts from articles in the journal for any purpose that respects the moral rights of authors, provided that the source is fully acknowledged. As a courtesy, the consent of authors of such material should be obtained directly from the author.

Authorization to reproduce items for other than personal use, as stated above, is granted by the National Research Council of Canada provided that the copyright fee of $7.00 per copy is paid directly to CANCOPY, One Yonge Street, Suite 1900, Toronto, ON M5E 1E5, Canada.

*The above permission does not extend to other kinds of copying, such as copying for general distribution, for advertising, or promotional purposes, for creating new collective works, or for resale. For such copying, arrangements must be made with the publisher, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

© Conseil national de recherches du Canada 1999. Tous droits réservés.
Il est interdit de reproduire, de sauvegarder ou de transmettre quelque partie de la présente publication dans quelque format ou de quelque façon sans le consentement écrit de l'éditeur, à l'exception des conditions suivantes.

Le Conseil national de recherches du Canada accorde aux particuliers le droit de faire des copies individuelles pour leur usage personnel. Toute personne peut reproduire de courts extraits des articles de la Revue pour autant que les droits moraux de l'auteur soient respectés et que la mention de provenance soit clairement indiquée. Il est de convenance de demander le consentement de l'auteur avant de faire mention de son travail.

On obtiendra l'autorisation de reproduire des articles à d'autres fins que l'usage personnel (tel qu'on l'a décrit ci-dessus) auprès du Conseil national de recherches du Canada pour autant que des redevances de droits d'auteurs de 7,00 $/copie soient versées au CANCOPY, 1, rue Yonge, bureau 1900, Toronto, ON M5E 1E5, Canada.

*La permission ci-dessus ne s'applique pas à la reproduction à grande échelle, telle que la reproduction aux fins de distribution générale, publicitaire ou promotionnelle, de compilation de nouveaux ouvrages collectifs ou de revente. Dans de tels cas, il faudra ratifier une entente particulière avec l'éditeur, le Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Subscription information

The *Canadian Journal of Fisheries and Aquatic Sciences* (ISSN 0706-652X) is published monthly by the National Research Council of Canada. 1999 annual subscription rates (second class postage) for Canada are $578CAN institutional and $182CAN personal, for US & foreign $578US institutional and $182US personal. For air mail add $150US to foreign rate. Canadian customers please include 7% GST (business no. 12149 1807 RT 0275). Subscriptions are entered for the calendar year. Remittances should be made payable to the Receiver General for Canada credit NRC. Price for regular single issues: institutional, $75.00; personal, $25.00. Supplements will be priced separately. Special rates apply to members of Canadian scientific and engineering societies if they subscribe through their respective societies.

There are no refunds on cancelled subscriptions once delivery has begun. Claims for missing issues should be made within four months (N. America) or six months (outside N. America) following the date of publication. The publisher expects to supply missing issues only when losses have occurred in transit and when the reserve stock will permit. For change of address and subscription enquiries: Subscription Office, NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada; tel: 613-993-9084; fax: 613-952-7656; e-mail: research.journals@nrc.ca.

Microform backfiles are available from UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, U.S.A., and Micromedia Ltd., 20 Victoria St., Toronto, ON M5C 2N8, Canada.

The 1999 issues of the *Canadian Journal of Fisheries and Aquatic Sciences* are available on the Web. Pricing information can be found at

http://www.nrc.ca/cisti/journals/

Renseignements sur l'abonnement

Journal canadien des sciences halieutiques et aquatiques (ISSN 0706-652X) est une publication mensuelle du Conseil national de recherches du Canada. Le prix de l'abonnement annuel au Canada (courrier de deuxième classe) est de 578 $CAN pour les organismes et de 182 $CAN pour les particuliers. À l'étranger (y compris les É.-U.), le prix de l'abonnement est de 578 $US pour les organismes et de 182 $US pour les particuliers. L'expédition par avion exige des frais de 150 $US en sus du prix d'abonnement à l'étranger. Les clients du Canada doivent ajouter la TPS de 7% (numéro d'entreprise 12149 1807 RT 0275). Les abonnements sont pour l'année civile. Tout paiement doit être adressé au Receveur général du Canada, crédit du CNRC. Le prix par numéro est de 75 $ pour les organismes et de 25 $ pour les particuliers. Le prix des hors-série varie. Les membres des sociétés canadiennes de sciences et de génie bénéficient de taux préférentiels en s'abonnant via leurs sociétés respectives.

Aucun remboursement ne sera accordé ni aucune annulation acceptée une fois que les livraisons auront commencé. Les réclamations de numéros manquants devront être soumises dans les quatre mois (Amérique du Nord) ou dans les six mois (à l'extérieur de l'Amérique du Nord) suivant la date de publication. L'éditeur ne fournira de numéros manquants que si le document a été perdu pendant l'expédition et que si le stock le permet. Adressez tout changement d'adresse ou toute question quant à l'abonnement au : Bureau des abonnements, Presses scientifiques du CNRC, Conseil national de recherches Canada, Ottawa, ON K1A 0R6, Canada; téléphone : 613-993-9084; télécopieur : 613-952-7656; adresse électronique : research.journals@nrc.ca.

On peut obtenir les fichiers rétrospectifs sur **microforme** auprès de UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, É.-U., et auprès de Micromedia Ltée, 20 rue Victoria, Toronto, ON M5C 2N8, Canada.

Les numéros de 1999 de *Journal canadien des sciences halieutiques et aquatiques* sont accessibles sur le Web. On trouvera des renseignements sur les tarifs à

http://www.nrc.ca/cisti/journals/rjfr.html

First observations of tumor-like abnormalities (exophytic lesions) on Lake Michigan zooplankton

M. Omair, Henry A. Vanderploeg, David J. Jude, and Gary L. Fahnenstiel

Abstract: Tumor-like abnormalities (exophytic lesions) were found on a variety of planktonic calanoid copepods and cladocerans (*Diaptomus* spp., *Epischura lacustris*, *Limnocalanus macrurus*, *Polyphemus pediculus*, *Diaphanosoma* sp., and *Daphnia galeata mendotae*) collected from inshore (3-m depth contour) and offshore (100- to 110-m depth contours) stations of eastern Lake Michigan. The abnormalities, which were quite large relative to animal size and variable in shape, are documented in photographs. Abnormality incidences among species ranged between 0 and 72%. Predatory species of calanoids and cladocerans had higher incidences of tumors than herbivorous species. The abnormalities on some copepods were very similar to cysts described for calanoid copepods in Lago Maggiore, Italy, which like Lake Michigan is undergoing oligotrophication. The recent appearance of the lesions in Europe and North America may indicate an emerging global phenomenon that has a common cause.

Résumé : Des anomalies de type tumoral (lésions exophytiques) ont été observées chez divers copépodes calanoïdes et cladocères planctoniques (*Diaptomus* spp., *Epischura lacustris*, *Limnocalanus macrurus*, *Polyphemus pediculus*, *Diaphanosoma* sp. et *Daphnia galeata mendotae*) capturés à des stations du littoral (isobathe de 3 m) et du large (isobathes de 100 m et 110 m) dans l'est du lac Michigan. Les anomalies, qui étaient plutôt grosses par rapport à la taille des animaux et de taille variable, ont été photographiées. L'incidence de ces anomalies, d'une espèce à l'autre, allait de 0 % à 72 %. Les espèces prédatrices présentaient une plus forte incidence de tumeurs que les espèces herbivores. Les tumeurs présentes chez certains copépodes étaient très semblables aux kystes décrits chez des copépodes calanoïdes du lac Majeur, en Italie, qui comme le lac Michigan est en voie d'oligotrophisation. La récente apparition des lésions en Europe et en Amérique du Nord peut révéler la présence d'un phénomène qui se développe à l'échelle mondiale et dont la cause est la même partout.

[Traduit par la Rédaction]

Introduction

We found tumor-like abnormalities (exophytic lesions) on *Limnocalanus macrurus*, a large predacious copepod that is a dominant of the hypolimnion of offshore Lake Michigan (Vanderploeg et al. 1998), in the first archived sample that we examined from collections made during 1995–1998 in the food web and water-quality monitoring program of the Great Lakes Environmental Research Laboratory (GLERL). Subsequently, we found a high incidence of abnormalities in a variety of nearshore species in zooplankton samples collected by the Center for Great Lakes and Aquatic Sciences (CGLAS), University of Michigan, from the nearshore zone.

Despite extensive collections of zooplankton from all the Great Lakes and intensive collections in Lake Michigan, no such lesions have been reported for North American freshwaters. In fact, very few authors have claimed to have ever seen tumors or tumor-like abnormalities in zooplankton in the oceans or lakes. Crisafi and Crescenti (1975, 1977) found tumor-like anomalies in 40 species of copepods from

Received April 27, 1999. Accepted July 30, 1999.
J15115

M. Omair.[1] Center for Great Lakes and Aquatic Sciences, 2021 C.C. Little Building, University of Michigan, 501 East University Avenue, Ann Arbor, MI 48109-1090, U.S.A.
H.A. Vanderploeg. National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105, U.S.A.
D.J. Jude. Center for Great Lakes and Aquatic Sciences, 717 Dennison Building, University of Michigan, 501 East University Avenue, Ann Arbor, MI 48109-1090, U.S.A.
G.L. Fahnenstiel. National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Lake Michigan Field Station, 1431 Beach Street, Muskegon, MI 49441-1098, U.S.A.

[1]Author to whom all correspondence should be addressed. e-mail: momair@umich.edu

© 1999 NRC Canada

polluted seas of the world. Silina and Khudolei (1994) reported tumor-like anomalies on zooplankton, but no photographs or descriptions of the anomalies on the zooplankton from a polluted area of the Baltic were ever presented. Here, we document incidences of tumors in a preliminary study of samples from 1995 to 1999 and present photographs of the abnormalities to encourage zooplankton ecologists to examine their samples for such abnormalities to determine geographical distribution of the lesions and stimulate research as to what is causing the lesions. Moreover, we show that some of the lesions bear a striking resemblance to cysts reported by Manca et al. (1996) in their study of epibionts on calanoid copepods in Lago Maggiore, an Italian lake undergoing oligotrophication.

Methods

Zooplankton in GLERL's offshore Lake Michigan monitoring program were collected with a 0.5-m-diameter, 153-mm-mesh net towed vertically from 95 m to the surface. One sample each from 22 June 1995 at a 110-m-deep site 15 km west of Muskegon, Michigan, and from 25 June 1998 at a 100-m-deep site 21 km west of Grand Haven, Michigan, were examined. Zooplankton samples were narcotized with club soda and preserved in a sugar formaldehyde solution (Haney and Hall 1973).

Nearshore zooplankton samples were collected as part of a yellow perch (*Perca flavescens*) recruitment study at 3-m depth contours near Muskegon, Michigan. A push net (0.5-m diameter, 363-mm-mesh net) mounted in front of a small boat and a 0.75-m-diameter, 363-mm-mesh net towed behind the boat from near bottom to the surface were used to collect larval fish and zooplankton. We report on samples preserved in 70% ethyl alcohol that were collected on 7, 8, and 19 June 1998.

To count the preserved samples, we split the samples with a Folsom plankton splitter with the goal of examining about 1000 individual zooplankton. For some of the rare taxa (nearshore cladocerans), we examined the entire sample to observe as many individuals as possible. Results of counts at all dates were combined so that we could report an overall lesion frequency for nearshore samples collected in June 1998.

Results

Appearance of abnormalities

The abnormalities on *Limnocalanus* were very prominent and found on the prosome (Figs. 1*a* and 1*b*) or occasionally on the urosome. Some large abnormalities were highly multilobed (Fig. 1*a*) or more typically large and almost spherical, attached by a stalk-like structure to *Limnocalanus* (Fig. 1*b*). Adult copepods affected were young adults because by June, the old generation had disappeared and the new generation was appearing (Vanderploeg et al. 1998). This is also borne out by the lack of infestation by the suctorian epibiont *Tokophrya quadripartita*, which infests older adults (Vanderploeg et al. 1998). The large lesions on young (recently molted) adults may imply especially rapid growth of the abnormalities if we assume that they appeared after molting. Abnormalities on *Epischura lacustris*, although not as large relative to body size as those on *Limnocalanus*, were quite prominent. Abnormalities on *Diaptomus* (Figs. 1*c* and 1*d*) were variable in size and some were almost as large relative to body size as those seen on *Limnocalanus*. Abnormalities on three species of cladocerans, *Polyphemus pediculus*, *Diaphanosoma* sp., and *Daphnia galeata mendotae*, were quite prominent and found in a variety of locations on the carapace and even on the abdominal claw (Figs. 1*e*, 1*f*, and 1*g*).

Frequency of infestation

We found abnormalities at appreciable frequencies in a variety of copepods (*Epischura lacustris*, *Eurytemora affinis*, and *Diaptomus* spp.) and *Polyphemus* (Table 1) in the nearshore sample collected at Muskegon. Percent occurrence was quite high, especially for *Epischura* (71.4%). Although we found abnormalities on *Limnocalanus* in the June 1995 offshore sample, we found none in the offshore sample collected in June 1998. Interestingly, no abnormalities were found on any species other than *Limnocalanus* at offshore sites. No abnormalities were found on *Cyclops* spp. at any site.

Affected zooplankton species showed a variety of taxonomic diversity (both cladocerans and copepods), habitats, feeding habits, and life histories (Table 2). That *Epischura* and *Polyphemus* had the highest frequency of infestation in the nearshore zone and *Limnocalanus* was the only species affected in the offshore zone suggests that predatory calanoid copepods and cladocerans are more likely to be affected than herbivorous or omnivorous species. A preliminary examination of samples from 1999 showed that abnormalities were present at a variety of sites in the southern basin of Lake Michigan as well as Green Bay, where abnormalities were found on *Diaptomus* spp.

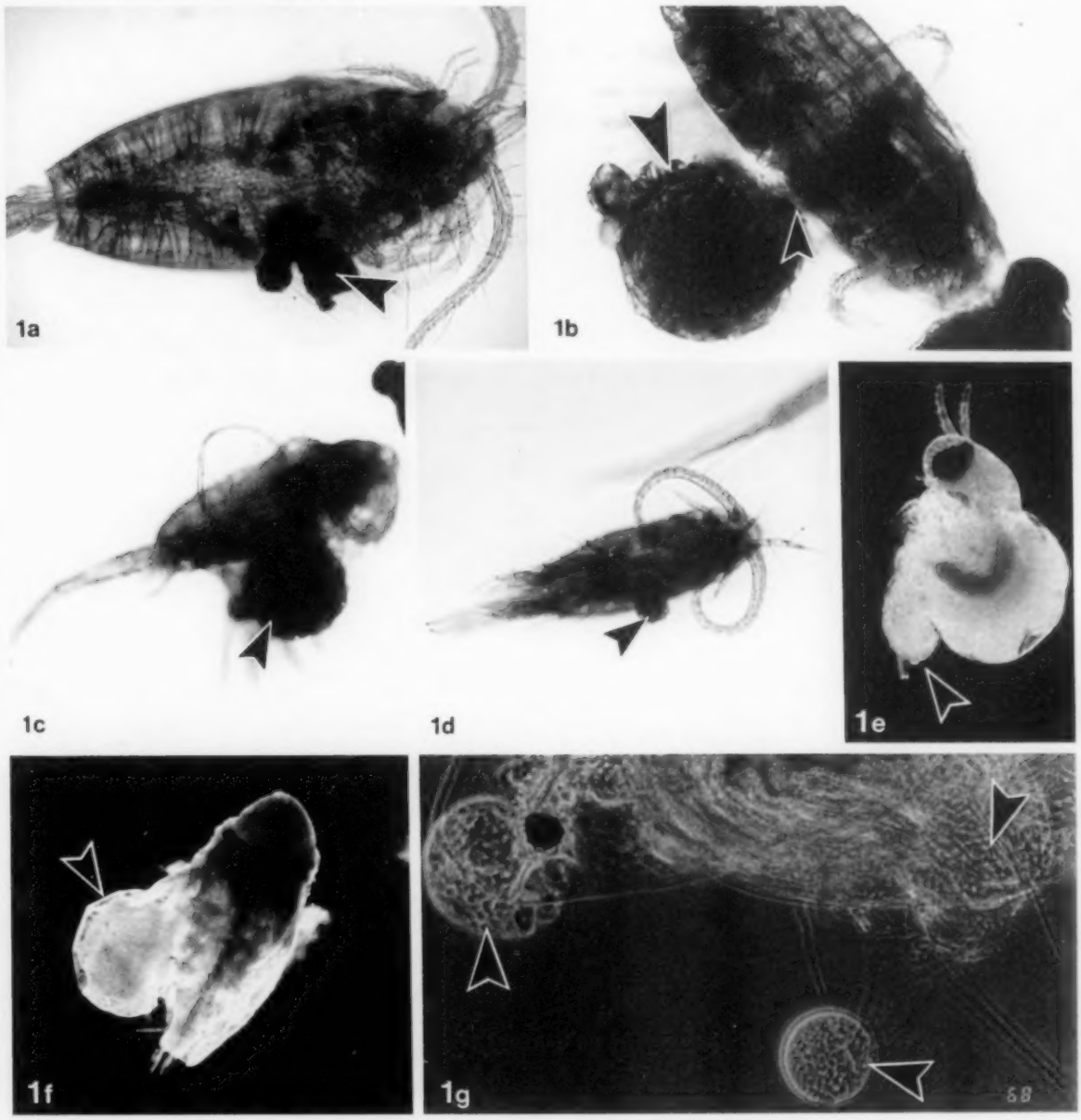
Discussion

A tumor, in general terms, is a swelling or protuberance; however, in medical terms, it implies a neoplasm, which implies growth of cells independent of the normal growth of the organism (Sparks 1972). Verification of a neoplasm can only be done at the cellular level, which we have not done here. Therefore, we have used the conservative description of tumor-like abnormality or exophytic lesion. Tumors and tumor-like abnormalities have been found in a variety of invertebrates: molluscs, arthropods (including insects and shrimp), flatworms, and cnidarians (Sparks 1972; Sparks and Lightner 1973; Overstreet and Van Devender 1978; Harshbarger 1997). However, reports of such abnormalities on crustaceans are rare and their causes and consequences to the affected organisms are obscure (Sparks and Lightner 1973; Overstreet and Van Devender 1978). Sparks and Lightner (1973) reported a tumor-like papilliform growth on the sixth abdominal segment of a brown shrimp that was tentatively diagnosed as a benign neoplasm. Overstreet and Van Devender (1978) reported an overgrowth (hamartoma) of muscle protruding through the sixth abdominal segment of postlarvae of two species of commercial shrimps. This nonneoplastic growth was thought to be an interaction between a pollutant and the normal growth process of the shrimp because the affected shrimp were found at a polluted site.

As far as zooplankton are concerned, Crisafi and Crescenti (1975, 1977) reported finding tumor-like anomalies in 40 species of copepods from polluted seas of the world. No frequencies of infestation or histological descrip-

© 1999 NRC Canada

Fig. 1. Appearance of tumor-like abnormalities (arrowheads) on zooplankton. (*a*) *Limnocalanus* (prosome length = 1.7 mm) with a large multilobed tumor on the ventral surface of the prosome between the swimming feet and mouthparts; (*b*) dorsal view of *Limnocalanus* (prosome length = 1.7 mm) with a large, nearly spherical abnormality (large arrowhead) attached by a stalk-like structure (small arrowhead) to the right side of the prosome; (*c* and *d*) tumor-like abnormalities on the ventral prosome of *Diaptomus* spp. (prosome length = 0.7 mm); (*e*) *Polyphemus pediculus* (body length = 0.7 mm); (*f*) dorsal view of *Diaphanosoma* sp. (body length = 0.9 mm); (*g*) *Daphnia galeata mendotae* (body length = 1.1 mm) with abnormalities on the helmet near the compound eye, abdominal claw, and carapace of the body.



tions were given. Some of the abnormalities that Crisafi and Crescenti (1975) photographed bear at least a superficial resemblance to the lesions that we describe. Silina and Khudolei (1994) found abnormalities on nauplii of cyclopoids and *Limnocalanus* in the Gulf of Finland (Baltic Sea) and nearby lakes. No descriptions of the gross anatomy or histology were reported. Frequencies of abnormalities were as high as 42–80% in a few samples from the southwest part of Neva Inlet, a polluted section of the Gulf of Finland. Further, Silina and Khudolei (1994) suggested that the con-

© 1999 NRC Canada

Table 1. Frequency of occurrence of tumor-like abnormalities among zooplankton species at offshore (100-m Grand Haven site in 1995 and 110-m Muskegon site in 1998) and inshore (3-m Muskegon site) sites in June 1995 and 1998 in eastern Lake Michigan.

	Offshore 1995		Offshore 1998		Inshore 1998	
Taxon	%	*N*	%	*N*	%	*N*
Cyclopoida						
Cyclops spp. copepodids and adults	0	214	0	237	0	49
Calanoida						
Diaptomus spp. copepodids and adults	0	602	0	798	9.3	236
Epischura lacustis copepodids and adults	—	0	0	12	71.4	899
Eurytemora affinis copepodids	—	0	—	0	4.8	41
Limnocalanus macrurus copepodids and adults	10	300	0	55	0	1
Cladocera						
Daphnia galeata mendotae	—	0	0	30	6.2	16
Diaphanosoma sp.	—	0	—	0	16.7	6
Polyphemus pediculus	—	0	—	0	33.3	3

Table 2. Ecology and life history traits of tumor-infested species.

Taxon	Habitat	Feeding habits	Life history
Copepoda			
Cyclops spp.	Inshore and offshore	Predators of microzooplankton and small crustaceans	Three generations per year?
Diaptomus spp.	Inshore and offshore	Omnivores with strong herbivorous tendencies	One or two generations per year; two of four species produce resting eggs
Epischura lacustris	Inshore and epilimnion	Omnivore, primarily predator of microzooplankton	One or two generations per year; resting eggs
Limnocalanus macrurus	Offshore hypolimnion	Omnivore, primarily predator of small crustaceans	One generation per year; no resting eggs
Cladocera			
Daphnia galeata mendotae	Inshore and offshore	Filter-feeder	Parthenogenic during summer; resting eggs
Diaphanosoma spp.	Inshore	Filter-feeder	Parthenogenic during summer; resting eggs
Polyphemus pediculus	Inshore	Predator of microzooplankton and small cladocerans	Two generations per year; parthenogenic during summer; resting eggs

Note: Accounts generalized from Balcer et al. (1984) and Vanderploeg et al. (1998).

centrations of cancer-causing chemicals such as polycyclic aromatic hydrocarbons and *N*-nitroso compounds as well as blue-green algal toxins, suspected carcinogens, should be investigated to see if they were the causal agents.

The spherical lesions on *Diaptomus* and *Limnocalanus* bear a striking resemblance to "cysts" in a paper describing epibionts on copepods in Lago Maggiore, a large Italian subalpine lake (Manca et al. 1996). Especially noteworthy is the similarity of the stalk-like attachment of the lesions in both studies. Up to 15% of the calanoid copepod *Eudiaptomus padanus* had cysts, and as appears to be the case from our limited data collected so far for Lake Michigan, the abnormalities were more common in summer. The cysts ranged between 35 and 140 mm with the average cyst being about 100 mm. The cysts were first observed in 1992 in this regularly monitored lake. Like Lake Michigan, Lago Maggiore is undergoing oligotrophication. Manca et al. (1996) speculated that the larger cysts might have had a negative impact on *Eudiaptomus padanus* because larger cysts were associated with a constriction of the body. Even if there was no direct harm to body tissues caused by the abnormalities, the large size of some of the Lake Michigan tumors would have interfered with swimming and feeding. Until we know more about the nature of the abnormalities and their effects on zooplankton fitness and health, we can only speculate as to what harm they are doing to the zooplankton and food web.

We suspect that occurrence of the abnormalities is a recent phenomenon in the Great Lakes, in general, and in Lake Michigan, in particular, because no abnormalities have been reported despite many monitoring and life history studies. Monthly zooplankton abundance was reported for the years 1971–1982 at nearshore sites out to the 40-m depth contour in a series of studies arising from the Cook Power Plant monitoring program in southeastern Lake Michigan (e.g., Evans 1986). No abnormalities were found on *Limnocalanus* in a lipid and life cycle study that carefully examined individual *Limnocalanus* monthly during 1987–1990 (Vanderploeg et al. 1998). Zooplankton abundance was also reported from the U.S. Environmental Protection Agency's (EPA) Lake Michigan Surveillance Plan for an extensive grid of offshore stations during the years 1983–1992. The U.S. Environmental Protection Agency collections were limited to the upper 20 m during spring and late summer; therefore, this program would have missed the nearshore zooplankton

© 1999 NRC Canada

as well as the deep-dwelling *Limnocalanus* during summer. No lesions were ever noticed (O. Johannsson, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, Ont., personal communication) in the extensive biomonitoring program on Lake Ontario during 1981 to the present.

Our findings along with those of Manca et al. (1996) and Silina and Khudolei (1994) suggest the intriguing possibilities that the abnormalities are a recent global phenomenon and that there is a common cause. Carcinogenic chemicals, ultraviolet radiation, viral or bacterial infections, or injury can cause tumors or tumor-like anomalies (e.g., Harshbarger 1997).

Histological examination of the tissues and cells of the tumor-like abnormalities now in progress will be helpful for determining whether they are neoplastic and whether a viral or bacterial agent is responsible. Whether or not the abnormalities are neoplastic, are caused by an environmental carcinogen, or are the result of an infectious agent, the consequences to the food web could be significant. We hope that this communication encourages further work. Further information is required to determine the first occurrence of the lesions in Lake Michigan, define their spatial distribution and seasonal timing, and ascertain their etiology.

Acknowledgements

This is GLERL publication No. 1136 and CGLAS publication No. 606. This research was conducted and supported by GLERL through a contract to M. Omair. Michigan Sea Grant (grant R/GLF-45 to D.J. Jude) provided funds for collection of nearshore zooplankton. We thank T. Rizki for useful discussions on tumors in invertebrates and for taking the photographs used in Fig. 1. We thank S. Hensler for helping sort zooplankton for tumors, N. Andresen and M. Edlund for taking photographs and reviewing the manuscript, P. Landrum and two anonymous reviewers for reviewing the manuscript, and J. Reid for a literature search. We thank M. Manca for contacting us to inform us of her work on cysts and B. Belonger and J. Dettmers for sending us zooplankton with lesions from Green Bay.

References

Balcer, M.D., Korda, N.L., and Dodson, S.I. 1984. Zooplankton of the Great Lakes — a guide to the identification and ecology of the common crustacean species. University of Wisconsin Press, Madison, Wis.

Crisafi, P., and Crescenti, M. 1975. Coseguenze delle attivita umane sullo zooplancton del Mare di Taranto. Boll. Pesca Piscic. Idrobiol. **30**: 207–218.

Crisafi, P., and Crescenti, M. 1977. Confirmation of a certain correlation between polluted areas and tumorlike conditions as well as tumours growths in pelagic copepods provening from numerous seas of the world. Rapp. Comm. Int. Mer Medit. **24**(10): 155.

Evans, M.S. 1986. Recent declines in zooplankton populations in the inshore region of Lake Michigan: probable causes and consequences. Can. J. Fish. Aquat. Sci. **43**: 154–159.

Haney, J.F., and Hall, D.J. 1973. Sugar-coated *Daphnia*: a preservation technique for *Daphnia*. Limnol. Oceanogr. **18**: 331–333.

Harshbarger, J.C. 1997. Invertebrate and cold-blooded vertebrate oncology. *In* Spontaneous animal tumors: a survey. *Edited by* L. Rossi, R. Richardson, and J. Harshbarger. Press Point di Abbiategrasso, Milano, Italy. pp. 41–53.

Manca, M., Beltrami, M., and Sonvico, D. 1996. On the appearance of epibionts on the crustacean zooplankton of a large subalpine lake undergoing oligotrophication (L. Maggiore, Italy). Mem. Ist. Ital. Irobiol. **54**: 161–171.

Overstreet, R.M., and Van Devender, T. 1978. Implication of an environmentally induced hamartoma in commercial shrimps. J. Invertebr. Pathol. **31**: 234–238.

Silina, N.I., and Khudolei, V.V. 1994. Tumorlike anomalies in planktonic copepods. Hydrobiol. J. **30**: 52–55.

Sparks, A.K. 1972. Invertebrate pathology — noncommunicable diseases. Chap. VII. Academic Press, New York.

Sparks, A.K., and Lightner, D.V. 1973. A tumor-like papilliform growth in the brown shrimp *Penaeus aztecus*. J. Invertebr. Pathol. **22**: 203–212.

Vanderploeg, H.A., Cavaletto, J.F., Liebig, J.R., and Gardner, W.S. 1998. *Limnocalanus macrurus* (Copepoda: Calanoida) retains a marine arctic lipid and life cycle strategy in Lake Michigan. J. Plankton Res. **20**: 1581–1597.

© 1999 NRC Canada

Relationship among adult body length, abundance, and ocean temperature for British Columbia and Alaska sockeye salmon (*Oncorhynchus nerka*), 1967–1997

Brian J. Pyper and Randall M. Peterman

Abstract: Body length of adult Pacific sockeye salmon (*Oncorhynchus nerka*) has decreased significantly in recent years. We used 69 time series of age-specific body-length data (1967–1997) for 30 sockeye salmon stocks from southern British Columbia to western Alaska to test hypotheses about the effects of oceanographic conditions and competition on growth rate of sockeye salmon. Using principal components analysis (PCA), we constructed a single time series (PC1) that represented the dominant pattern of variability in length-at-age shared among these stocks. Taking into account time trends and autocorrelation in residuals, we found that increases in total Gulf of Alaska sockeye abundance and increases in sea-surface temperature (SST) across the Gulf of Alaska were significantly associated with reduced adult body length. Abundance and SST together accounted for 71% of the variability in PC1. Although researchers have documented increases in both abundance of sockeye salmon and their food in the northeastern Pacific Ocean over the last few decades, it is possible that increased food was more than offset by increased sockeye abundance, leading to greater competition and reduced body size.

Résumé : Depuis quelques années, la longueur des saumons rouges (*Oncorhynchus nerka*) adultes du Pacifique baisse de façon notable. Nous avons utilisé 69 séries chronologiques de données sur la longueur du corps spécifique à l'âge (1967–1997) pour 30 stocks de saumon rouge, du sud de la Colombie-Britannique à l'ouest de l'Alaska, afin de vérifier les hypothèses concernant les effets des conditions océanographiques et de la compétition sur le taux de croissance du saumon rouge. À l'aide d'une analyse des composantes principales, nous avons construit une seule série chronologique (PC1) qui représentait le régime dominant de variabilité de la longueur selon l'âge commun à tous ces stocks. En tenant compte des tendances temporelles et de l'autocorrélation présente dans les résidus, nous avons découvert que les hausses de l'abondance totale du saumon rouge dans le golfe de l'Alaska et les augmentations de la température de la surface de la mer (SST) en travers du golfe de l'Alaska étaient associées de façon significative à la réduction de la longueur du corps chez les adultes. L'abondance et la SST prises ensemble représentaient 71 % de la variabilité dans la série PC1. Bien que les chercheurs signalent des augmentations de l'abondance du saumon rouge et de leurs sources de nourriture depuis quelques décennies, il est possible que l'augmentation de la quantité de nourriture ait été plus que contrebalancée par la hausse de l'abondance du saumon rouge, ce qui a accru la compétition et réduit la taille des saumons.

[Traduit par la Rédaction]

Introduction

Over the last two decades, body size of adult Pacific sockeye salmon (*Oncorhynchus nerka*) has decreased dramatically, thereby reducing the reproductive potential of spawners and the contribution per fish harvested to economic benefits. Recently, Pyper et al. (1999) found widespread positive covariation in adult body length among 31 geographically diverse sockeye salmon stocks from British Columbia (B.C.) and Alaska. This covariation indicates that these stocks encounter similar growing conditions when rearing in the Gulf of Alaska. Current evidence suggests that competition for food and ocean conditions such as sea-surface temperature (SST) may drive temporal variation in adult body size (e.g., Peterman 1984; Rogers and Ruggerone 1993; McKinnell 1995; Cox and Hinch 1997). However, much of this past research focused on individual stocks or regions. In this paper, we used the dominant pattern of variability in adult body length shared by numerous B.C. and Alaska sockeye stocks to more broadly test hypotheses about the effects of oceanographic conditions and competition on growth rate of sockeye salmon.

Received July 13, 1999. Accepted August 18, 1999.
J15245

B.J. Pyper and R.M. Peterman. School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

[1]Author to whom all correspondence should be addressed.
e-mail: peterman@sfu.ca

Methods

Data

We used 69 time series of body length at a given adult age sampled from 30 sockeye stocks from four geographically distinct areas or "regions" in B.C. (Fraser River and Skeena River) and

© 1999 NRC Canada

Alaska (Upper Cook Inlet and Bristol Bay) (see Pyper et al. 1999 for details). Because of the strong positive covariation in body length among these stocks, we constructed a single time series representing the dominant pattern of shared variation in length-at-age, as follows. First, each of the 69 length series was standardized to have zero mean and unit variance. Next, a single "aggregated" data series was created for each of our four regions and each age-class by averaging across the population-specific length series for a given year. These aggregated lengths better depict the shared variability or common "signal" of environmental influences specific to each age-class and region than would data series for individual stocks. We used these aggregated data series to give each regional age-class equal weighting in the principal components analysis (PCA) described below. This procedure generated 13 aggregated length series (see fig. 3 of Pyper et al. (1999), except the Copper River stock was omitted because of missing data), three for Fraser River (ages 1.1, 1.2, and 1.3), two for Skeena River (ages 1.2 and 1.3), and four for each of Cook Inlet and Bristol Bay sockeye stocks (ages 1.2, 1.3, 2.2, and 2.3). Finally, we used an unrotated PCA to extract the dominant pattern of covariation (PC1) among the 13 length series. This PCA was restricted to the 31 years (1967 to 1997) for which data were available for all 13 series.

We generated indices of sockeye salmon abundance and SST that might reflect conditions during periods when most of the variability in final body size occurs (i.e., the final year or months at sea, as shown by Rogers and Ruggerone (1993), McKinnell (1995), and Pyper et al. (1999)). These indices were also consistent with the area of overlap in ocean distributions of B.C. and Alaska sockeye (French et al. 1976), which roughly encompasses the Alaskan Gyre and is occupied by sockeye salmon from North America but not Asia (French et al. 1976). We used total ocean abundance of maturing North American sockeye as an index of competition, based on annual adult recruits (catch plus escapement) summed across the major B.C. (Fraser, Skeena, and Nass River) and Alaska stocks (Copper River, Cook Inlet, and Bristol Bay), which together account for the vast majority of sockeye abundance in the Gulf of Alaska (see Peterman et al. (1998) for details).

To reflect ocean conditions that might affect growth, we generated two separate time series of annual SST deviations from the long-term mean. We used monthly SST data (°C) on a 5° × 5° latitude–longitude grid (D. Cayan and L. Riddle, Scripps Institute of Oceanography, La Jolla, Calif., personal communication) across the above-noted area of general overlap in ocean distributions of B.C. and Alaska sockeye, which was a polygon enclosing the latitude lines along 45°N from 170°W to 150°W, along 50°N from 175°W to 145°W, and along 55°N from 155°W to 140°W. Deviations were computed for a given grid cell and month by subtracting its long-term mean SST for 1947 through 1997. These deviations were then averaged to create two time series, each corresponding to a different aggregation of months preceding the return (generally in July) of sockeye salmon to their rivers: (*i*) winter months (November through February), and (*ii*) a combination of winter and spring months (November through June). Climatic forcing during winter months is thought to be an important determinant of ocean productivity in the subsequent spring and summer (e.g., Brodeur and Ware 1992), while in spring months, maturing sockeye are feeding and growing at high rates before and during migrations back to their natal streams. Most results reported here are for November–February SST, but November–June results were similar, as noted below.

Statistical analyses

We used multiple regression to examine relationships among the dominant pattern of covariation for length (PC1), total sockeye abundance in millions of fish (A), and oceanographic temperature (SST) for either November–February or November–June:

$$(1) \qquad PC1 = a + b_1 A + b_2 SST + \varepsilon$$

where a, b_1, and b_2 are parameters and ε is the residual variation. Because these time series contained obvious increasing or decreasing time trends, we used two approaches. First, we computed regressions using the original data. Second, we calculated deviations from a linear time trend fit to each data series and repeated the regressions using those detrended data to test for effects at shorter time scales.

Residuals from the multiple regressions fit using ordinary least squares (OLS) were approximately normally distributed with constant variance but contained positive autocorrelation. Such autocorrelation leads to underestimation of standard errors of regression parameters, creating more rejections of the null hypothesis of zero slope than is warranted (Bence 1995). We therefore used the following procedure to account for autocorrelation, which is equivalent (see Griffiths et al. 1993) to Bence's (1995) recommended "bias-corrected iterated two-stage generalized least squares with a pre-test." After fitting eq. 1 using OLS, we calculated a Durbin–Watson statistic to test for significant positive autocorrelation in the residuals at $\alpha = 0.5$ (yes, 0.5, not 0.05). Because this test rejected the null hypothesis of zero lag-1 autocorrelation in our regressions, we simultaneously computed maximum likelihood estimates of the lag-1 autocorrelation coefficient and regression parameters.

Results

The PCA of the 13 aggregated length-at-age series yielded three principal components (PCs) with eigenvalues greater than 1, accounting for 65%, 11%, and 8% of the total variance, respectively. The clear dominant component, PC1, correlated strongly with all 13 length series (r ranging from 0.64 to 0.89; average $r = 0.81$). Thus, the time series for PC1 effectively captured a dominant pattern of variation in body length shared by B.C. and Alaska sockeye from 1967 to 1997 (Fig. 1A), which included a general declining trend ($r = -0.62$ for PC1 vs. time).

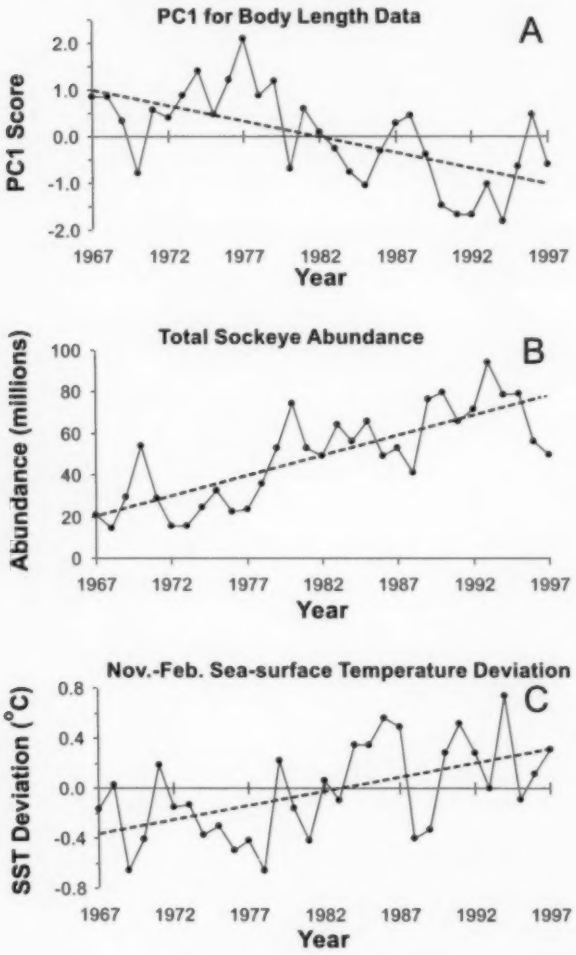
The multiple regression with the original data (Fig. 2) was highly significant (multiple $r^2 = 0.71$, $P << 0.001$), as was the estimated slope on abundance ($b_1 = -0.03 \pm 0.005$ [±1 SE], $P << 0.001$). The slope on SST was also significant ($b_2 = -0.72 \pm 0.29$, $P = 0.014$). Partial r^2 values were 0.56 for abundance and 0.18 for SST.

Although there was moderate multicollinearity between abundance and November–February SST ($r = 0.44$), it did not affect interpretation of results. There was no obvious "ridge" of data points (Fig. 2) from which a few chance outliers might seriously bias the estimate of one of the slopes. Furthermore, multicollinearity tends to *inflate* standard errors of parameter estimates (Griffiths et al. 1993), making P values too high. However, because we already rejected both hypotheses (that $b_1 = 0$ and $b_2 = 0$) with P values < 0.05, this problem is not an issue here.

Because time trends in the original data (Fig. 1) might possibly confound interpretation of results, we also did the multiple regression using detrended data. Both abundance and SST were significant for this regression ($b_1 = -0.035 \pm 0.007$, $P << 0.001$, and $b_2 = -0.87 \pm 0.31$, $P = 0.004$). Overall, the multiple $r^2 = 0.54$, $P << 0.001$, and multicollinearity was negligible ($r = 0.04$). Partial r^2 values were 0.48 for abundance and 0.23 for SST.

© 1999 NRC Canada

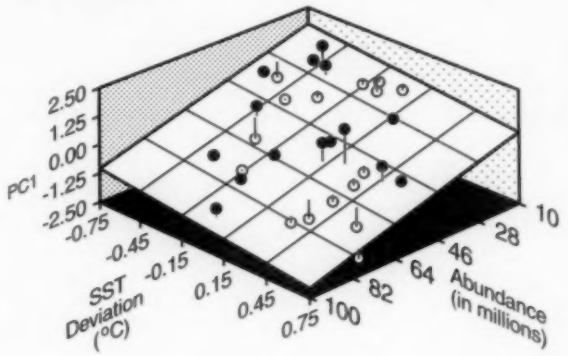
Fig. 1. (A) Scores for the dominant principal component (PC1) of length-at-age data for B.C. and Alaska sockeye salmon. Lower values of PC1, for example, reflect the tendency toward reduced body length that is shared among these stocks. (B) Total annual adult recruits (catch plus escapement) of the major B.C. and Alaska sockeye stocks. (C) Average yearly winter (November through February) sea-surface temperature (SST) deviations in °C from the long-term (1947–1997) mean for those months (6.8°C) for the northeast Pacific Ocean over the region where distributions of B.C. and Alaska sockeye salmon overlap. Dotted lines are the fitted linear time trends used to detrend the data.



In addition, because November–February and November–June SST data were highly correlated ($r = 0.86$), all results for the latter were similar to those for November–February, with the exception that SST was not significant in either the multiple regression with original data ($P = 0.11$) or with detrended data ($P = 0.08$).

Finally, we added an interaction term ($+ b_3A \cdot \text{SST}$) to the multiple regression in eq. 1, but this term had a negligible effect (for b_3, $P = 0.11$; multiple $r^2 = 0.73$ compared with 0.71 for eq. 1). Abundance still had a strong significant effect ($P << 0.001$) but November–February SST did not ($P = 0.76$).

Fig. 2. Multiple regression with observed data (1967–1997) shown as residuals from the regression plane (positive residuals as solid dots, negative residuals as open dots; multiple $r^2 = 0.71$, $P << 0.001$). The vertical axis is the dominant principal component (PC1) of length-at-age data for adult B.C. and Alaska sockeye salmon. Independent variables are from the last year of ocean life: abundance is total annual adult recruits (catch plus escapement in millions) of the major B.C. and Alaska sockeye stocks; SST deviation is yearly deviation in winter sea-surface temperature (for November through February) from the long-term mean, in °C, for the northeast Pacific Ocean, averaged over the region where distributions of B.C. and Alaska sockeye salmon overlap.



Discussion

Our results indicate that increases in total sockeye abundance and November–February SST are associated with reduced adult body length of both B.C. and Alaska sockeye salmon. Abundance and SST together account for 71% of the variability in PC1 of body length among 30 major sockeye stocks of the northeastern Pacific Ocean during 1967–1997. Furthermore, abundance appears to have a much greater effect on body size than temperature. Its partial r^2 was considerably larger (0.56 vs. 0.18), and when data in each series were transformed into standard deviation units so that slopes were in the same units, the standardized slope for abundance (–0.68) was greater than the slope for SST (–0.27). This indicates that for each standard deviation increase, abundance had about 2.5 times the contribution to reducing adult body size as did November–February SST.

Furthermore, both abundance and SST were significantly related to the dominant pattern of variability in body length at both long and short time scales (i.e., in the original as well as in detrended data). Thus, although it is possible that relationships among the original data might be coincidental because of their co-occurring time trends (i.e., that some other omitted variable actually explains the trend in PC1), the evidence in support of the effects of abundance and SST was strengthened by their very similar slopes and P values in both the original data analysis and the analysis once time trends were removed.

© 1999 NRC Canada

These findings are consistent with past research that showed reduced body size of either B.C. or Bristol Bay sockeye in years when abundance of the latter was high (Peterman 1984; Rogers and Ruggerone 1993; McKinnell 1995). Over the period analyzed here, Bristol Bay sockeye accounted for 64% of total adults, with Fraser stocks and the remaining B.C. and Alaska stocks each accounting for only 18%. Moreover, the temporal pattern of total sockeye abundance (Fig. 1B) more closely resembled Bristol Bay abundance ($r = 0.94$) than that of Fraser ($r = 0.56$), Skeena ($r = 0.52$), or Cook Inlet stocks ($r = 0.49$). Thus, it seems likely that density-dependent effects on growth for all stocks were largely determined by competitive interactions with the abundant Bristol Bay stocks. This density-dependent effect has important international policy implications: environmental conditions and management actions that increase abundance of sockeye stocks from Bristol Bay, Alaska, may indirectly reduce body size and hence the economic value of each British Columbia sockeye salmon caught, as well as eggs produced by each female B.C. sockeye spawner. To our knowledge, such effects have not been taken into account by managers.

Our finding that adult body size decreases as SST increases is consistent with results of Cox and Hinch (1997) and Hinch et al. (1995) for Fraser River sockeye salmon. They speculated that unusually high SST could reduce growth directly by increasing energetic costs of metabolism or indirectly by reducing temperature-defined ocean distributions of fish and consequently increasing inter-stock competition. In contrast, Rogers and Ruggerone (1993) found a positive relationship between SST at Kodiak (a coastal location) and adult body size of Bristol Bay sockeye. This opposite result may be due to the use of different SST indices or patterns of variability in body size of Bristol Bay sockeye not captured by our PC1. Although we found that smaller body size was associated with higher SST, we cannot speculate reliably about effects of future global warming outside the range of past-observed SST because SST reflects the net result of complex interacting processes of ocean dynamics such as wind-driven upwelling or downwelling, circulation patterns, and vertical stratification (Brodeur and Ware 1992). Therefore, further research is necessary to understand the mechanisms underlying relationships between SST and body size of sockeye salmon.

Although several authors have documented increased secondary productivity in the northeastern Pacific Ocean in recent decades (e.g., zooplankton and squid (Brodeur and Ware 1992, 1995)), which should improve growth rates for sockeye salmon, abundance of sockeye recruits also increased (Fig. 1B). We therefore hypothesize that increased food supply was more than offset by increased sockeye abundance, which resulted in greater competition and smaller body size in recent years. This increased competition among sockeye could have been exacerbated by greater competition with other species such as pink salmon (*O. gorbuscha*), which also increased in abundance recently (Brodeur and Ware 1995). However, the very similar parameter estimates using original and detrended data noted above suggest that within-species competitive interactions among sockeye salmon dominate.

Our work extends previous research on changes in adult body size of sockeye salmon. For example, Hinch et al. (1995) used data for only one stock, Early Stuart (Fraser River, B.C.) sockeye salmon, and SST at one location, Ocean Station P. Also, other researchers related body size for several stocks to either SST (Cox and Hinch 1997) or abundance (Peterman 1984), but not both. In contrast, our results apply to the dominant pattern of covariation in body length among 5 age-classes and 30 sockeye stocks from southern B.C. to western Alaska, and our independent variables were region-wide SST and total abundance of most major sockeye stocks in the Gulf of Alaska.

Although our multiple regression accounted for a large portion (71%) of the shared variability in adult body size, our results do not imply that all ages and stocks were equally affected by abundance and SST. Indeed, distinct region-specific and age-specific patterns of variability in length are also evident for the sockeye populations examined here (Pyper et al. 1999). Such patterns most certainly reflect differences in the ocean distributions of groups of B.C. and Alaska sockeye salmon during periods critical to growth and thus reflect differences in their competitive interactions and exposure to other factors influencing growth.

Acknowledgments

We are extremely grateful to the numerous biologists and technicians in various management agencies who gathered, processed, and provided the time series of data analyzed here. We also thank Dan Ware, Carl Schwarz, and Ian Bercovitz for useful discussions and Carl Schwarz, Michael Bradford, Judith Anderson, and Brice MacGregor for comments on the manuscript. Brice MacGregor also kindly assisted with the statistical analysis. Funding was provided by a grant to R.M. Peterman from the Natural Sciences and Engineering Research Council of Canada.

References

Bence, J.R. 1995. Analysis of short time series: correcting for autocorrelation. Ecology, **76**: 628–639.

Brodeur, R.D., and Ware, D.M. 1992. Long-term variability in zooplankton biomass in the subarctic Pacific Ocean. Fish. Oceanogr. **1**: 32–38.

Brodeur, R.D., and Ware, D.M. 1995. Interdecadal variability in distribution and catch rates of epipelagic nekton in the Northeast Pacific ocean. *In* Climate change and northern fish populations. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 329–356.

Cox, S.P., and Hinch, S.G. 1997. Changes in size at maturity of Fraser River sockeye salmon (*Oncorhynchus nerka*) (1952–1993) and associations with temperature. Can. J. Fish. Aquat. Sci. **54**: 1159–1165.

French, R., Bilton, H., Osaka, M., and Hartt, A. 1976. Distribution and origin of sockeye salmon (*Oncorhynchus nerka*) in offshore waters of the North Pacific Ocean. Int. North Pac. Fish. Comm. Bull. **34**.

Griffiths, W.E., Hill, R.C., and G.G. Judge. 1993. Learning and practicing econometrics. Wiley, New York.

Hinch, S.G., Healey, M.C., Diewert, R.E., Thomson, K.A., Hourston, R., Henderson, M.A., and Juanes, F. 1995. Potential ef-

© 1999 NRC Canada

fects of climate change on marine growth and survival of Fraser River sockeye salmon. Can. J. Fish. Aquat. Sci. **52**: 2651–2659.

McKinnell, S. 1995. Age-specific effects of sockeye abundance on adult body size of selected British Columbia sockeye stocks. Can. J. Fish. Aquat. Sci. **52**: 1050–1063.

Peterman, R.M. 1984. Density-dependent growth in early ocean life of sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **41**: 1825–1829.

Peterman, R.M., Pyper, B.J., Lapointe, M.F., Adkison, M.D, and Walters, C.J. 1998. Patterns of covariation in survival rates of British Columbian and Alaskan sockeye salmon (*Oncorhynchus nerka*) stocks. Can. J. Fish. Aquat. Sci. **55**: 2503–2517.

Pyper, B.J., Peterman, R.M., Lapointe, M.F., and Walters, C.J. 1999. Patterns of covariation in length and age at maturity of British Columbia and Alaska sockeye salmon (*Oncorhynchus nerka*) stocks. Can. J. Fish. Aquat. Sci. **56**: 1046–1057.

Rogers, D.E., and Ruggerone, G.T. 1993. Factors affecting marine growth of Bristol Bay sockeye salmon. Fish. Res. **18**: 89–103.

© 1999 NRC Canada

Effects of an erroneous natural mortality rate on a simple age-structured stock assessment

William G. Clark

Abstract: The abundance of many stocks is estimated by fitting an age-structured model to catch-at-age and relative abundance data from the commercial fishery and scientific surveys. The natural mortality rate used in the model is usually estimated externally and its value is uncertain. An erroneous natural mortality rate will bias the stock size estimates obtained by fitting the model and will also bias the yield calculations that are done to choose a harvest rate and recommend quotas. This paper describes the general features of both effects by analyzing a simple age-structured model fitted to artificial data. It is shown that an erroneous natural mortality rate mainly affects the estimates of fishing mortality and hence abundance but not the estimates of age-specific selectivity. Errors in estimated abundance and target harvest rate are always in the same direction, with the result that, in the short term, extremely high exploitation rates can be recommended (unintentionally) in cases where the natural mortality rate is overestimated and historical exploitation rates in the catch-at-age data are low. A conservative (low) estimate of natural mortality can avoid that danger. Long-term yield under either an F_{MSY} or $F_{35\%}$ strategy is not very sensitive to error in natural mortality rate unless it is grossly underestimated.

Résumé : On estime l'abondance de nombreux stocks en appliquant un modèle structuré selon l'âge à des données de capture selon l'âge et d'abondance relative provenant de la pêche commerciale et des relevés scientifiques. Le taux de mortalité naturelle utilisé dans le modèle est habituellement estimé indépendamment, et sa valeur est incertaine. Un taux de mortalité naturelle erroné biaisera les estimations de la taille du stock obtenues par l'application du modèle, de même que les calculs du rendement réalisés pour établir un taux de capture et les quotas recommandés. Dans cet article, on décrit les caractéristiques générales de ces deux effets en analysant un modèle simple structuré selon l'âge appliqué à des données artificielles. On montre qu'un taux de mortalité naturelle erroné influe principalement sur les estimations de la mortalité par pêche et donc de l'abondance, mais pas sur les estimations de la sélectivité en fonction de l'âge. Les erreurs dans l'abondance estimée et le taux de capture cible vont toujours dans le même sens, ce qui fait que, à court terme, des taux d'exploitation extrêmement élevés peuvent être recommandés (de façon non intentionnelle) dans des cas où le taux de mortalité naturelle est surestimé et où les taux d'exploitation passés établis à partir des données de capture selon l'âge sont faibles. On peut se prémunir contre ce danger en établissant une estimation conservatrice (valeur basse) de la mortalité naturelle. Une production à long terme selon une stratégie fondée sur F_{PME} ou $F_{35\%}$ n'est pas très sensible à l'erreur touchant le taux de mortalité naturelle, à moins que ce paramètre ne soit largement sous-estimé.

[Traduit par la Rédaction]

Introduction

The last two decades have seen the development of a variety of modern age-structured methods for estimating stock size from a time series of catch-at-age data and some index of relative abundance, most often a scientific survey (Fournier and Archibald 1982; Collie and Sissenwine 1983; Deriso et al. 1985; Methot 1989). All of them are elaborations of the simple DeLury or depletion model (Leslie and Davis 1939; DeLury 1947) in that they infer absolute population size from the effect of known absolute removals on relative abundance. For example, if a brief fishery that removes X tons is observed to reduce relative abundance by 10%, the exploitable stock must have amounted to $10X$ tons before the fishery started. Modern age-structured methods make the same kind of estimate for every year-class that appears in the catch-at-age data, but in doing so, they must account for the effects of natural mortality and age-specific selectivity (survey and (or) commercial) on the abundance and catchability of each year-class at each age.

Age-specific selectivity can usually be estimated reasonably well along with other parameters in the course of fitting a model, but not natural mortality. The model can usually be fitted equally well with any of a wide range of fixed natural mortality rates. Standard practice is therefore to estimate natural mortality externally and use that estimate as a fixed value when fitting the model. The difficulty is that natural mortality is notoriously difficult to estimate by any means, so the external estimates are usually weak.

Unfortunately the stock size estimates obtained by fitting a model are quite sensitive to the chosen value of natural mortality. The effect of error in the natural mortality rate on stock size estimates obtained by cohort analysis (Pope 1972) has been investigated by a number of authors (Mertz and Myers 1997 and references cited therein), but relatively little attention has been given to its effect on stock size estimates

Received October 16, 1998. Accepted April 16, 1999.
J14832

W.G. Clark. International Pacific Halibut Commission, University of Washington, P.O. Box 95009, Seattle, WA 98145-2009, U.S.A. e-mail: wgclark@u.washington.edu

© 1999 NRC Canada

obtained by modern assessment methods. The difference presumably results from the nature of the two procedures. Cohort analysis consists of a formula by which historical abundance is back-calculated, so general expressions for the effects of various errors can be derived. Modern assessment methods rely on numerical function minimizers to locate the parameter estimates, so it is not a straightforward task to predict the effect of an error.

Thompson (1994) developed a simple deterministic model in which natural mortality and selectivity were indeterminate and considered the problem of setting a single catch quota when absolute abundance was estimated from a single survey. This is somewhat different from the usual age-structured stock assessment in which absolute abundance is estimated by fitting a model to a time series of relative abundance data. Schnute and Richards (1995) simulated internal estimates of natural mortality along with stock size and other parameters of a conventional stochastic age-structured model fitted to a time series of reliable relative abundance data. Their results confirmed that natural mortality is poorly estimated, with the possible exception of cases where the catch-at-age data extend back to the beginning of the fishery. Neither paper considered the effect of error in a fixed external estimate of natural mortality on long-term yield.

The purpose of this paper is to track the effect of an error in natural mortality through the whole assessment cycle from fitting an age-structured model through choosing a target harvest rate and calculating a recommended quota. Some of the parameter values and functional forms were chosen to approximate the fishery for Pacific halibut (*Hippoglossus stenolepis*), but the aim was to develop some general insights into the assessment process in its simplest form rather than to conduct a sensitivity analysis of the actual Pacific halibut assessment.

Effect on stock size estimates

The model investigated here is a variant of the classic separable model of fishing mortality first published by Doubleday (1976). In this paper the age-specific selectivity (partial recruitment factor) is treated as a measure of availability rather than relative vulnerability as in the standard version. This modification simplifies some of the algebra and provides nearly the same results. Specifically, let $a = 1,\ldots, A$ be age, coded so that the youngest age group in the data has index 1, $y = 1,\ldots, Y$ be year, $C_{y,a}$ be catch in number of age a fish in year y, $N_{y,a}$ be number of age a fish alive at the beginning of year y, F_y be instantaneous rate of fishing mortality in year y on fish fully recruited to the fishery, s_a be commercial fishery selectivity (partial recruitment factor) at age a, $H_{y,a} = s_a(1 - \exp(-F_y))$ be exploitation or harvest rate on age a fish in year y, and M be instantaneous rate of natural mortality. The timing of fishing and natural mortality is not important for this analysis. For simplicity, it will be assumed here that the catch is taken during a brief fishery at the beginning of the year so that $C_{y,a} = H_{y,a}N_{y,a} = s_a(1 - \exp(-F_y))N_{y,a}$ and natural mortality follows the fishery so that $N_{y+1,a+1} = N_{y,a}(1 - s_a(1 - \exp(-F_y))\exp(-M)$.

If the catches are generated according to the model and measured without error (or treated that way), the only model parameters are the $\{F_y\}$, $\{s_a\}$, and M, and the only data are the catches in the catch-at-age table. Experience has shown that even with M fixed externally, this model usually fits the catch-at-age data well but the estimated fishing mortality rates and stock sizes are extremely sensitive to small changes in the catch data, and as a result highly variable.

To stabilize the estimates, the model is always fitted to one or more series of relative abundance data as well as the catch-at-age data. The relative abundance data determine the level of total mortality by showing the decline in abundance of individual year-classes. The estimate of fishing mortality is then obtained as the difference between the apparent rate of total mortality and the chosen rate of natural mortality, and finally the stock size estimates are determined by the fishing mortality rates and the catches. The weak link in this chain is obviously the natural mortality rate, which is usually not known very well. Any error in the chosen value of natural mortality, within limits, will simply be offset by a compensating error in the fishing mortality rates (and stock sizes) so that the correct total mortality is preserved.

The ability of the fitted model to closely reproduce the catch-at-age and relative abundance data over a range of natural mortality rates can be exploited to develop analytical approximations for the errors in other parameter estimates induced by an error in the natural mortality rate. This section of the paper does that for fits of the separable model to artificial, error-free catch-at-age and relative abundance data. The results show, at least approximately, what bias in the parameter estimates can be expected on average when the model is fitted to stochastic data.

Consider first the simple case where the full-recruitment fishing mortality rate is the same for all years, so $F_y = F$ throughout, and the relative abundance data are commercial catch per effort in number, a set of values proportional to $s_aN_{y,a}$. Suppose also that the selectivity schedule is asymptotic, so that $s_a = 1$ for two or more of the oldest age groups, and the fitting algorithm forces the estimated selectivities to be asymptotic as well. (For fitting purposes, it is essential that at least two of the final selectivities be 1. If only the final selectivity is 1, there is an indeterminacy between the selectivities and total mortality and the model cannot be fitted.)

Some basic results will be obtained for this simple case and then extended to other cases. Throughout the derivations, a circumflex over a symbol will denote the (numerical) estimate of a parameter or derived quantity, e.g., $\hat{F}_y$ will denote the fitted estimate of F_y. Even though not fitted internally, the working value of natural mortality will be denoted $\hat{M}$. The error $(\hat{M} - M)$ will de denoted δ, so $\hat{M} = M + \delta$.

The derivations will rely on two features of the model fits. First, it will be assumed that the model can be fitted in the first place, i.e., that the objective function has a unique minimum for each of a range of natural mortality rates and the minimizer locates it. Second, it will be assumed that the model fit does in fact reproduce the catch-at-age and relative abundance data closely for each of a range of natural mortality rates. These are the conditions that define the cases of interest in this analysis. Together, they imply that if a given set of parameter estimates can be shown to reproduce the data, then by virtue of uniqueness, they must be the ones that the minimizer would locate.

© 1999 NRC Canada

Fully recruited age groups

The relative abundance data for fully recruited age groups will reflect the true value of total mortality $Z = F + M$, and the model fit will capture that feature, i.e., $\hat{Z} = Z$. Hence

$$\hat{F} + \hat{M} = F + M$$

$$\hat{F} + M + \delta = F + M$$

$$\hat{F} = F - \delta.$$

So the error in the fitted estimate of full-recruitment fishing mortality will be equal and opposite to the error in the natural mortality rate. Note that if δ is positive, it must be less than F. If it were not, $\hat{M}$ would exceed Z and the model could not be fitted.

Both the model population and the real population must generate the given catch-at-age and relative abundance data. With a constant F, the exploitation rate will be the same for all fully recruited age groups in all years and equal to $H = 1 - \exp(-F)$ or the real population and $\hat{H} = 1 - \exp(-\hat{F}) = 1 - \exp(-(F - \delta))$ for the model population. Hence

$$(1) \qquad \hat{H}\hat{N}_{y,a} = HN_{y,a} = C_{y,a}$$

$$\hat{N}_{y,a} = (H/\hat{H})N_{y,a}.$$

These estimates will clearly reproduce the catches exactly and also the relative abundance data (because $\hat{Z} = Z$), so by uniqueness, they are the fitted estimates. As a corollary, fully recruited age groups in the real population will also appear as fully recruited in the model population.

Partially recruited age groups

For partially recruited age groups, what the model has to fit is the ratio of successive relative abundance indexes for each year-class as it passes through the population, i.e., $(s_{y+1,a+1}N_{y+1,a+1})/(s_{y,a}N_{y,a})$. Leaving out the year subscript on the understanding that we are dealing with a particular year-class, the required condition on the estimates is

$$(2) \qquad \frac{\hat{s}_{a+1}\hat{N}_{a+1}}{\hat{s}_a\hat{N}_a} = \frac{s_{a+1}N_{a+1}}{s_aN_a}$$

$$\frac{\hat{s}_{a+1}[\hat{N}_a(1 - \hat{s}_a\hat{H})\exp(-(M + \delta))]}{\hat{s}_a\hat{N}_a}$$

$$= \frac{s_{a+1}[N_a(1 - s_aH)\exp(-M)]}{s_aN_a}$$

$$\frac{\hat{s}_a}{s_a} = \frac{\hat{s}_{a+1}}{s_{a+1}} \frac{(1 - \hat{s}_a\hat{H})}{(1 - s_aH)} \exp(-\delta).$$

Let $E_a = \hat{s}_a/s_a$, so $\hat{s}_a = E_a s_a$ and eq. 2 can be written as

$$E_a = E_{a+1} \frac{(1 - E_a s_a \hat{H})}{(1 - s_aH)} \exp(-\delta).$$

Solving for E_a gives

$$(3) \qquad E_a = \frac{E_{a+1}}{(1 - s_aH)\exp(\delta) + E_{a+1}s_a\hat{H}}.$$

Because the selectivities are asymptotic, $\hat{s}_A = s_A = E_A = 1$ and the fitted selectivities $\{\hat{s}_a = E_a s_a\}$ can be calculated recursively with eq. 3.

Values of the selectivities calculated in this way will match the relative abundance data, meaning the relative magnitudes of the index for all age groups. In particular (with $\hat{s}_A = s_A = 1$),

$$(4) \qquad \frac{\hat{s}_a\hat{N}_a}{\hat{N}_A} = \frac{s_aN_a}{N_A}$$

$$\hat{N}_a = \frac{s_a}{\hat{s}_a} \frac{\hat{N}_A}{N_A} N_a$$

$$\hat{N}_a = \frac{s_a}{\hat{s}_a} \frac{H}{\hat{H}} N_a.$$

The corresponding calculated catch at age a is

$$\hat{C}_a = \hat{H}_a\hat{N}_a = (\hat{s}_a\hat{H})\left(\frac{s_a}{\hat{s}_a} \frac{H}{\hat{H}} N_a\right) = s_aHN_a = C_a.$$

Even with an error in $\hat{M}$, therefore, the fitted model can reproduce the catch-at-age and relative abundance data exactly. Like Thompson's (1994) model, this form of the simple age-structured model is a case where the natural mortality rate and the selectivity (i.e., availability) parameters are formally indeterminate. This means that even with perfect data, M cannot be estimated internally and fitting the model requires an external estimate.

General features of the biased fits

Some examples will illustrate the effect of error in the chosen value of natural mortality on model fits. Deterministic catch-at-age and relative abundance data for these examples were generated using the availability form of the separable model described above. True selectivity at age was calculated with a function that has the form of the left limb of a normal density and whose two parameters are the age at full recruitment (a_f, coded age 9) and the age at half recruitment (a_h, coded age 4):

$$s_a = \exp\left\{-(\log 2)(a_f - a)^2/(a_f - a_h)^2\right\} \text{ for } a < a_f$$

and

$$s_a = 1 \text{ for } a \geq a_f.$$

While the true age-specific selectivities were calculated with this parametric function, they were estimated as individual parameters when the model was fitted, and the last three (out of 15) were fixed at 1. Parameter estimates obtained by fitting the model numerically were equal to the values obtained with the computing formulas derived above.

Table 1 shows the fitted estimates for a range of true natural mortality rates when fishing mortality $F = 0.15$ and the model is fitted with $\hat{M} = 0.20$. For example, for true $M = 0.10$ (case 1), $\delta = \hat{M} - M = +0.10$, fishing mortality is estimated to be $\hat{F} = F - \delta = 0.05$, and the abundance of fish in the fully recruited age groups is estimated to be $(H/\hat{H}) = 2.86$ times the true abundance, a sizable error. The estimate of (coded) age 1 abundance is even further off, 4.01 times

© 1999 NRC Canada

Table 1. True parameter values and fitted estimates for a range of true natural mortality rates M when fishing mortality is constant at $F = 0.15$ and the model is fitted with $\hat{M} = 0.20$.

Quantity	Case 1	Case 2	Case 3	Case 4	Case 5
True natural mortality rate M	0.10	0.15	0.20	0.25	0.30
Chosen natural mortality rate $\hat{M}$	0.20	0.20	0.20	0.20	0.20
Error $\delta = \hat{M} - M$	+0.10	+0.05	0.00	–0.05	–0.10
True fishing mortality rate F	0.15	0.15	0.15	0.15	0.15
Estimated fishing mortality rate $\hat{F}$	0.05	0.10	0.15	0.20	0.25
Estimated selectivity at (coded) age:					
1	0.12	0.14	0.17	0.20	0.23
2	0.20	0.23	0.26	0.29	0.32
3	0.31	0.34	0.37	0.40	0.43
4	0.44	0.47	0.50	0.53	0.55
5	0.60	0.62	0.64	0.66	0.68
6	0.75	0.77	0.78	0.79	0.80
7	0.88	0.89	0.90	0.90	0.91
8	0.97	0.97	0.97	0.97	0.98
9+	1.00	1.00	1.00	1.00	1.00
Estimated age at 50% recruitment	4.38	4.20	4.00	3.79	3.57
Estimated age at 100% recruitment	8.92	8.96	9.00	9.05	9.10
Estimated age 9+ abundance as a multiple of true numbers	2.86	1.46	1.00	0.77	0.63
Estimated age 1 abundance as a multiple of true numbers	4.01	1.72	1.00	0.66	0.47

Note: The true selectivities at age are listed under case 3.

the true abundance, because for partially recruited fish the underestimate of fishing mortality is compounded by the underestimate of selectivity. An underestimate of natural mortality by the same amount ($\delta = -0.10$, case 5) produces an underestimate of abundance by about 40% (the factor 0.63). For an error of a given magnitude $|\delta|$ in the natural mortality rate, the relative error in the abundance estimates is much larger in the case of an overestimate than for an underestimate.

The reasons for this behavior are apparent from the formula for the relative error:

$$\frac{\hat{N} - N}{N} = \frac{(H/\hat{H})N - N}{N}$$

$$= \frac{(1 - \exp(-F))}{(1 - \exp(-(F - \delta))} - 1$$

$$\approx \frac{\delta}{(F - \delta)} \text{ for small } F - \delta > 0.$$

Obviously, a large F will swamp even a large δ, but even a small δ can result in a sizable error when F is small, and more so if δ is positive. The worst case is a positive δ only a little smaller than F. The true value of M has no effect on the error.

The selectivity estimates are much less sensitive to error in the natural mortality rate because only a small adjustment to the true values is needed to fit the data. Rewriting eq. 2 with $H = 1 - \exp(-F)$ and $\hat{H} = 1 - \exp(-F - \delta))$ gives

$$\left(\frac{\hat{s}_a}{s_a}\right) = \left(\frac{\hat{s}_{a+1}}{s_{a+1}}\right) = \frac{(1 - \hat{s}_a(1 - \exp(-(F - \delta))))}{(1 - s_a(1 - \exp(-F)))\exp(\delta)}$$

$$\approx \left(\frac{\hat{s}_{a+1}}{s_{a+1}}\right)\frac{\exp(-\hat{s}_a(F - \delta))}{\exp(-s_a F)\exp(\delta)}$$

$$\approx \left(\frac{\hat{s}_{a+1}}{s_{a+1}}\right)\exp(-\delta(1 - s_a)).$$

Again, by virtue of the asymptotic selectivities $(\hat{s}_A/s_A) = 1$, so

$$\left(\frac{\hat{s}_a}{s_a}\right) \approx \prod_{i=a}^{A-1}\exp(-\delta(1 - s_i)) = \exp\left(-\delta\sum_{i=a}^{A-1}(1 - s_i)\right).$$

For an age group that is 50% recruited, $(1 - s_i)$ will be 0.5 and because selectivity is normally increasing rapidly at this point, the remaining terms in the summation will sum to perhaps another 0.5, for a total of around 1. The ratio $(\hat{s}_a/s_a)$ will therefore be on the order of $\exp(-\delta) \approx 1 - \delta$ and the relative error in $\hat{s}_a$ will be on the order of δ, which cannot be much more than 10%. For the smaller selectivities the relative error will be larger, but the absolute error will still be modest, as in the examples shown. Note that here, too, the size of the error does not depend on the true value of M.

Variable fishing mortality

All of the analytical results derived thus far have relied on the fishing mortality rate F remaining constant from year to year and therefore from age to age (for fully recruited age groups), but they also hold approximately when the $\{F_y\}$ are allowed to vary, so long as there is no strong trend or contrast in the series and the error $\delta = \hat{M} - M$ is not too large relative to the mean of the $\{F_y\}$. In this situation, if $\bar{F}$ is the mean of the true values $\{F_y\}$, the mean of the estimates $\{\hat{F}_y\}$ is $\bar{\hat{F}} \approx \bar{F} - \delta$, and the annual estimates of harvest rates are

© 1999 NRC Canada

$$\hat{H}_y \approx H_y\left(\frac{\hat{\bar{H}}}{\bar{H}}\right) = H_y\left(\frac{1-\exp(-\bar{F})}{1-\exp(-(\bar{F}-\delta))}\right)$$

For fully recruited fish the estimated numbers-at-age are $\hat{N}_{y,a} \approx \left(\frac{\bar{H}}{\hat{\bar{H}}}\right) N_{y,a}$, as in the case of constant fishing mortality, and because eq. 2 is not at all sensitive to F, eqs. 3 and 4 also hold (to a close approximation) for partially recruited age groups when the estimated selectivities are calculated analytically with $\bar{F}$ in place of a constant F.

The correspondence is close but not exact because when there is error in $\hat{M}$ and variance in the $\{F_y\}$, the analytical approximations do not track the $\{Z_y\}$ exactly, and the minimizer redistributes the total fishing mortality slightly so that the fitted $\{\hat{Z}_y\}$ will track better. That is easy to do when δ is negative because then, $\hat{\bar{F}} > \bar{F}$ and the $\{\hat{Z}_y\}$ tend to overshoot the $\{Z_y\}$. Redistributing a small amount of fishing mortality requires only a small relative change in any of the $\{\hat{F}_y\}$, so they can be made to both track the $\{Z_y\}$ and produce the catches very closely, to within a few percent.

When δ is positive, and particularly when $(\bar{F}-\delta)$ is near zero, the problem is harder and the best solution is to create a small amount of fishing mortality over and above $(\bar{F}-\delta)$, even at the cost of exceeding $\bar{Z}$ slightly. As a purely empirical finding based on simulations with the specific model studied here, when the $\{F_y\}$ were drawn from a uniform distribution running from 0.5 $\bar{F}$ to 1.5 $\bar{F}$ and δ was positive, the fitted mean fishing mortality rate was approximately $\hat{\bar{F}} \approx (\bar{F}-\delta) + 0.05\delta = \bar{F} - 0.95\delta$. So, for example, with $\bar{F} = 0.15$ and $\delta = +0.10$, the estimate averaged $\hat{\bar{F}} = 0.055$ instead of 0.050 as in the case of constant F. This is a small absolute difference, but it can have a significant effect on the estimates of abundance because they are inversely proportional to $\hat{\bar{H}}$. In this example the estimated abundance of fully recruited fish is 2.86 times the true value when F is constant (Table 1), but on average only 2.60 times when the $\{F_y\}$ vary in the manner stated.

A related peculiarity of the fits obtained when $(\bar{F}-\delta)$ is near zero and the $\{F_y\}$ vary is that the selectivities of the older age groups, including some fully recruited ones, are made to sag slightly so as to offset the excess of $\hat{\bar{Z}}$ over $\bar{Z}$. This is an exception to the general rule that the selectivity of older fish is well estimated even when the natural mortality rate is in error.

With the adjustment $\hat{\bar{F}} = \bar{F} - 0.95\delta$ for $\delta > 0$ (and the approximate selectivities calculated with that value), the analytical approximations do a good job of predicting the numerically fitted estimates when fishing mortality varies.

Trends in fishing mortality

When the true fishing mortality rates contain a major trend, or a contrast between periods, and the natural mortality rate is wrong, the model fits to deterministic data always show large residuals with very clear trends. A good fit is simply impossible. This suggests that in principle the natural mortality rate should be estimable in these cases, but in practice, other sources of variance tend to obscure the patterns resulting from lack of fit. At any rate, these cases are beyond the scope of this paper, which is concerned with situations where many good fits are possible.

Effect on yield calculations and harvest rates

Many stocks are managed by applying a target harvest rate to estimated present stock size to arrive at a recommended upper limit for the quota. The target harvest rate is based either on an estimate of the spawner–recruit relationship (F_{MSY}) or on a target value of spawning biomass per recruit, typically 35 or 40% of the unfished value ($F_{35\%}$ or $F_{40\%}$; Clark 1991). Error in the working value of natural mortality affects these calculations in three ways as follows.

(*i*) Biased estimate of spawner productivity

For a given set of life history parameters, the optimum harvest rate for a stock depends mostly on the slope of the spawner–recruit relationship at the origin, which is the productivity of spawners at very low stock levels. In most stocks the bulk of spawners are fully recruited and the recruits are the youngest age group in the model (coded age 1). In this situation, an error in the natural mortality rate will lead to an estimate of the slope equal to the true value multiplied by

$$\frac{(\hat{N}_1/\hat{N}_f)}{(N_1/N_f)} = \frac{(\hat{N}_1/N_1)}{(\hat{N}_f/N_f)} = (s_1/\hat{s}_1)$$

(by eq. 4). For example, an overestimate of natural mortality lowers the estimated selectivities of partially recruited age groups and elevates the estimated numbers, resulting in an overestimate of the slope parameter of the spawner–recruit relationship.

(*ii*) Biased estimate of recruit productivity

Other things being equal, yield and spawning biomass per recruit decrease as the natural mortality rate increases. In the case of yield calculations based on an estimated spawner–recruit relationship, an overestimate of natural mortality will result in an underestimate of recruit productivity, which to some extent should offset the associated overestimate of spawner productivity. When a reference fishing mortality rate like $F_{35\%}$ is calculated, an error in natural mortality will affect both the unfished and fished values of spawning biomass per recruit, but in practice, $F_{35\%}$ is usually quite sensitive to the natural mortality rate. Both target rates will also be affected by the distorted selectivities, but not very much.

(*iii*) Translation from a target harvest rate to a real harvest rate

A catch quota recommendation is calculated by applying the target harvest rate to the numbers in the model population, but then the catch is taken from the real population where the numbers are different, so the real harvest rate is different. Let $\hat{H}^*$ denote the estimated target (full-recruitment) harvest rate, C^* the recommended quota, and $\tilde{H}_1$ the resulting real harvest rate in the first year. Then, for fully recruited fish:

$$C_f^* = \hat{H}^* \hat{N}_f = \hat{H}^*(H/\hat{H})N_f \text{ so } \tilde{H}_1 = \hat{H}^*(H/\hat{H}).$$

© 1999 NRC Canada

Table 2. Age-specific parameters used in yield calculations.

Age	Coded age	Selectivity	Mean weight	Maturity
6	1	0.17	16.83	0.03
7	2	0.26	16.83	0.06
8	3	0.37	16.83	0.12
9	4	0.50	20.65	0.21
10	5	0.64	23.36	0.34
11	6	0.78	26.07	0.50
12	7	0.90	28.78	0.68
13	8	0.97	31.49	0.84
14	9	1.00	34.20	0.96
15	10	1.00	36.92	1.00
16	11	1.00	39.63	1.00
17	12	1.00	42.34	1.00
18	13	1.00	45.05	1.00
19	14	1.00	47.76	1.00
20	15	1.00	50.47	1.00
21	16	1.00	53.18	1.00
22	17	1.00	55.89	1.00
23	18	1.00	58.60	1.00
24	19	1.00	61.31	1.00
25	20	1.00	64.02	1.00

When $\hat{M}$ is an overestimate, $\hat{H}$ is an underestimate, so an overestimate of natural mortality will generally result in a real harvest rate higher than the target. In cases where H is low, $H/\hat{H} \approx F/(F-\delta)$ and even a small overestimate can cause a large overshoot.

If $\hat{H} = \hat{H}^*$, then $\tilde{H}_1 = H$ and the fishery will continue indefinitely at a harvest rate of $\tilde{H}_1$. That is, the equilibrium harvest rate for a given nominal target fishing mortality rate F^* (e.g., F_{MSY} or $F_{35\%}$) and a given natural mortality estimate $\hat{M}$ is the one for which the estimate of average fishing mortality in the catch-at-age data $\hat{F}$ is equal to the estimate of the nominal target rate $\hat{F}^*$. And since $\hat{F} = F - \delta$, the real equilibrium rate $\tilde{F}_{eq}$ is

$$(5) \qquad \tilde{F}_{eq} = \hat{F}^* + \delta.$$

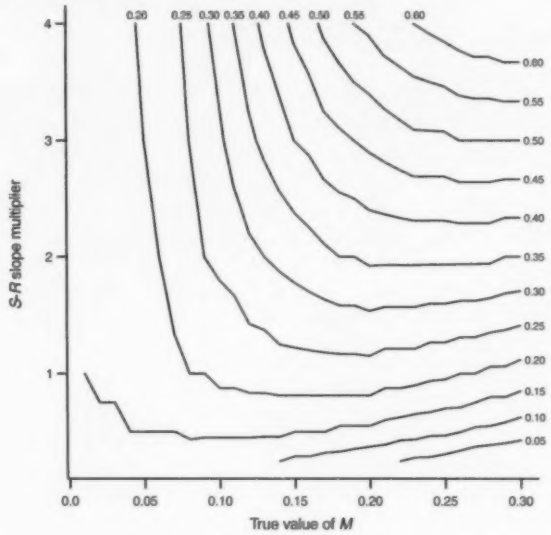
This is a stable equilibrium because $\tilde{H}_1$ will be greater than H when $\hat{H} < \hat{H}^*$ and will be less than H when $\hat{H} < \hat{H}^*$. If natural mortality is severely underestimated, $\tilde{F}_{eq}$ can be negative, meaning that estimated fishing mortality $\hat{F}$ exceeds $\hat{F}^*$ even when real fishing mortality is nil, so there is no equilibrium.

Calculated effects for a specific case

The consequences of error in the natural mortality rate were calculated for a specific life history similar to Pacific halibut, using the analytical approximations derived above for the case of variable fishing mortality. The advantages of using the approximations are that they save a great deal of time and they avoid the computational problems that can occasionally arise during attempts to fit the model numerically, even to artificial data.

The life history parameters used in the calculations are shown in Table 2. They correspond roughly to a longline fishery for Pacific halibut with no minimum size limit. The age-specific selectivities are the ones described above.

Fig. 1. Contours of true F_{MSY} as a function of true natural mortality rate M and a multiplier of the slope of the spawner–recruit (S-R) relationship used in this study.



Recruitment levels for yield calculations were based on a Beverton–Holt spawner–recruit relationship:

$$R = \alpha S/(1+\beta S)$$

where R is recruitment in number at coded age 1 and S is parent spawning biomass. The slope at the origin α was set to 0.12 recruit per unit of spawning biomass, a value for which $F_{MSY} = F_{35\%}$ for $M = 0.20$. The scale parameter β has no effect other than to scale all the calculated yields.

For the life history parameters and spawner–recruit relationship used here, the true values of F_{MSY} and $F_{35\%}$ depend on the true value of M as follows:

M	0.05	0.10	0.15	0.20	0.25	0.30
F_{MSY}	0.17	0.21	0.23	0.23	0.21	0.18
$F_{35\%}$	0.14	0.16	0.19	0.23	0.29	0.36

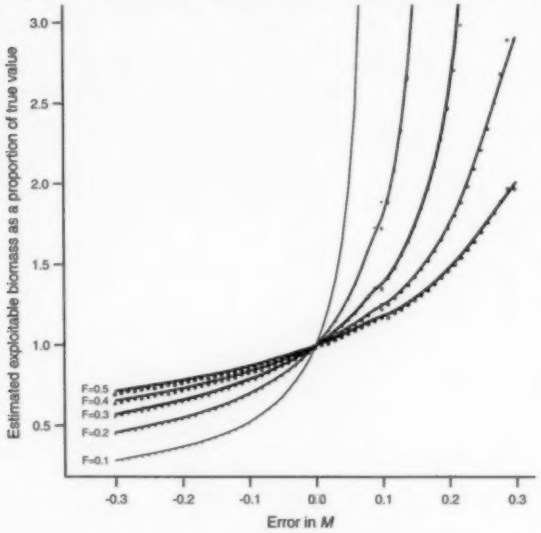
Evidently, F_{MSY} is much less sensitive to the true natural mortality rate than is $F_{35\%}$ in this case. Figure 1 shows how the true value of F_{MSY} varies when the slope of the spawner–recruit relationship is varied by a factor of 4 above and below the central value. At the lower end of the spawner productivity range, the true value of F_{MSY} is more sensitive to the slope parameter than to M, while at the upper end, it is more sensitive to M than to the slope parameter. Within a factor of 2 of the central value, the slope parameter is much more important. The true value of $F_{35\%}$ is not affected by the slope of the spawner–recruit relationship.

Short-term effects

The error in estimated stock size depends on the average level of fishing mortality in the catch-at-age data and on the error in natural mortality but not on the true value of natural mortality. These errors were computed over a grid of average fishing mortality rates $0 \le \bar{F} \le 0.5$, true natural mortality rates $0 < M \le 0.3$, and estimated natural mortality rates

© 1999 NRC Canada

Fig. 2. Estimated exploitable biomass as a proportion of the true value as a function of the error in estimated natural mortality for average (historical) fishing mortality rates $\bar{F}$ of 0.1, 0.2, 0.3, 0.4, and 0.5. The thickness of the points and lines is an index of $\bar{F}$.



$0 < \hat{M} < \min(0.3, \bar{F} + M)$. Even at the higher levels of $\bar{F}$, and even when the error in $\hat{M}$ is 0.10 or less, there are substantial errors in the biomass estimates (Fig. 2), but they are generally less than 25% when $\bar{F} \geq 0.3$. At lower levels of $\bar{F}$, even small errors in $\hat{M}$ result in large errors, e.g., a two- or three-fold overestimate when $\bar{F} = 0.1$ and $\hat{M} = M + 0.05$.

The errors in estimates of F_{MSY} and $F_{35\%}$ depend on the true value of natural mortality and the error in estimated natural mortality but not (significantly) on the average level of fishing mortality in the catch-at-age data. Overestimates of M result in overestimates of the target rates (Fig. 3). Estimates of F_{MSY} are somewhat less sensitive to error in the estimate of natural mortality than are estimates of $F_{35\%}$. For example, with true $M = 0.20$, the estimate $\hat{F}_{MSY}$ varies from about 0.18 to 0.27 for values of $\hat{M}$ within ±0.10 of the true value, while the estimate $\hat{F}_{35\%}$ varies from about 0.15 to 0.40. In fact, the estimates $\hat{F}_{MSY}$ are quite serviceable whenever the error in $\hat{M}$ is less than 0.10.

Error in the estimate of the target harvest rate compounds the error in the stock size estimate, with the result that real harvest rates differ from the nominal targets by even more than the stock size estimates differ from the true values (Fig. 4). In particular, the real short-term harvest rate under either an F_{MSY} or $F_{35\%}$ strategy can easily be two or three times the true value of F_{MSY} when the average fishing mortality rate in the catch-at-age data is low and the natural mortality rate is overestimated by as little as 0.05. Underestimates of natural mortality result in much smaller errors, so in the short term, it is preferable to adopt a conservative (i.e., low) estimate of natural mortality if the average fishing mortality rate is low, or may be low.

Equilibrium conditions

Under either harvest strategy, the real harvest rate will tend toward the equilibrium given by eq. 5. This limits the range of potential errors, but even at equilibrium the real harvest rate under either strategy can range from about a quarter to twice the desired value for errors of 0.10 or less in the estimate of natural mortality (Fig. 5). The F_{MSY} strategy is somewhat better behaved across the whole range of true natural mortality rates and errors than the $F_{35\%}$ strategy.

In terms of yield, both strategies perform quite well for errors up to ±0.10 in the natural mortality rate, with F_{MSY} again more consistent across the whole range considered (Fig. 6). The only region of somewhat poor performance is where the true natural mortality is low (less than 0.20) and it is substantially underestimated. But the region of good performance is quite wide, so a conservative (low) estimate of natural mortality will still perform well so long as it is not overly conservative.

Like the actual yield, the estimate of maximum sustainable yield (MSY) is close to the true value for errors up to ±0.10 in the natural mortality rate (Fig. 7). Depending on the value used, the impression may be of a smaller, more productive stock or a larger, less productive stock, but the potential long-term surplus production comes out about the same. The robustness of the MSY estimate only holds in the vicinity of the equilibrium fishing mortality rate; MSY calculations based on estimates at much higher or lower fishing mortality rates can differ greatly from the correct value, in either direction.

Other model formulations

All of the analysis above refers to the availability version of the separable model of fishing mortality fitted to commercial catch per effort. In fact, the vulnerability version of the separable model is more common, and it is almost always fitted to a survey index of abundance. In many fisheries the separable model of fishing mortality cannot be used at all because commercial selectivity is known or suspected to be variable. This section will extend the analysis to cover those cases.

Standard separable model

If age-specific selectivity is treated as relative vulnerability rather than availability, age-specific harvest rates for a brief fishery are calculated as $H_a = 1 - \exp(-s_a F)$ and commercial catch per effort is a set of values proportional to $\{H_a N_a\}$ rather than $\{s_a N_a\}$. The selectivities $\{\hat{s}_a\}$ that will reproduce the relative abundance data in this case when fishing mortality is constant and $\hat{M} \neq M$ can be calculated with computing formulas analogous to eqs. 2 and 3, and it can be shown that these selectivities and the corresponding estimated numbers-at-age will also generate the catch-at-age data exactly. The same result can be obtained when the Baranov catch equation is used. The form of the catch equation will not affect the general features and conclusions of the analysis performed with the availability version of the model, so they apply to these cases.

While it is possible to choose a catch equation and relative abundance index that will result in exact agreement between the model predictions and the data even when $\hat{M}$ is in error, inconsistencies are also possible. For example, if the standard separable model is used to generate the catches but the index of abundance is the one used above (proportional

© 1999 NRC Canada

Fig. 3. Contours of the estimated value of (*a*) F_{MSY} and (*b*) $F_{35\%}$ (at equilibrium) as a function of true and estimated natural mortality. The 1:1 line (labeled "*M* error = 0") is the locus of the true value for a given natural morality rate. In the region marked with asterisks, there is no equilibrium.

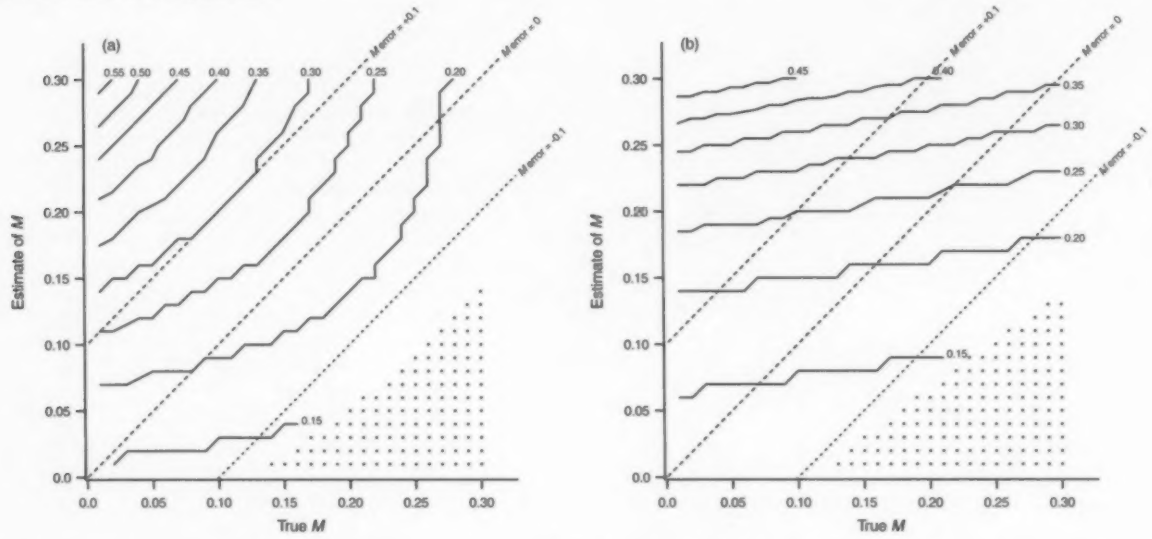
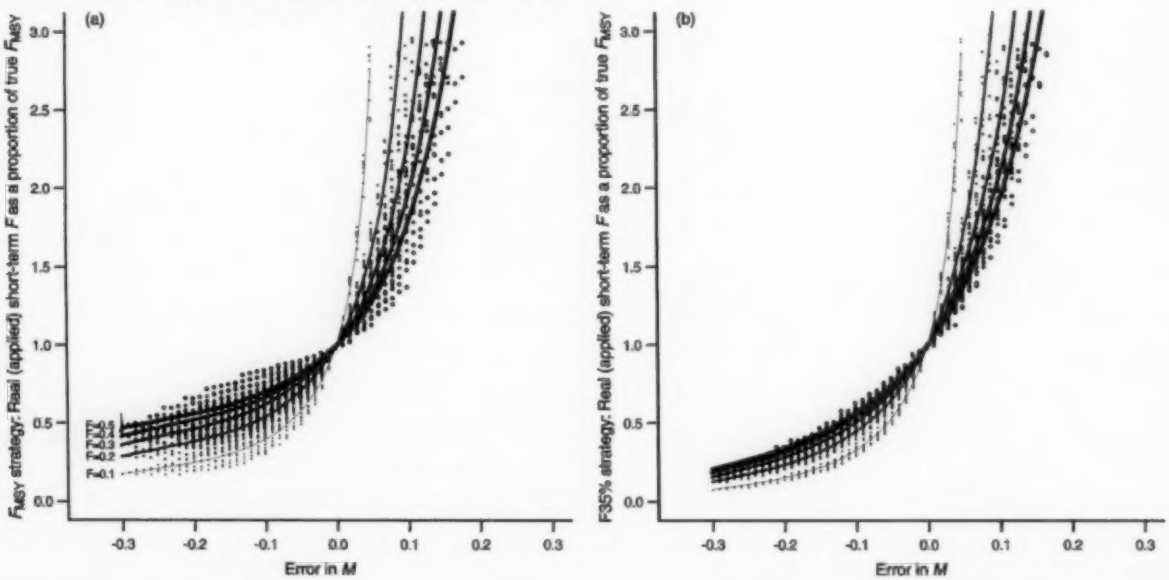


Fig. 4. Real short-term fishing mortality $\tilde{F}_1$ relative to F_{MSY} as a function of error in estimated natural mortality when the target harvest rate is (*a*) F_{MSY} and (*b*) $F_{35\%}$ for the cases plotted in Fig. 2.



to s_aN_a), it is not possible to reproduce both data sets exactly when there is an error in the natural mortality rate. The relative abundance data and the catch-at-age data require slightly different selectivities, and the fitted selectivities are a compromise. They are a very good compromise in that the model fit is still good to several digits, but it is not exact.

Survey index of abundance

Suppose that fishing mortality is constant and the separable model has been fitted exactly to commercial catch per effort as above, the index being proportional to s_aN_a. Now, a survey index with different selectivities, say t_aN_a, is brought into the fit. By eq. 4,

$$t_aN_a = t_a\left(\frac{\hat{s}_a}{s_a}\right)\left(\frac{\hat{H}}{H}\right)\hat{N}_a = \hat{t}_a\hat{N}_a$$

so with (normalized) survey selectivities $\{\hat{t}_a\}$ the existing fit will reproduce the survey index exactly, and therefore, those are the fitted survey selectivities, even if commercial catch

© 1999 NRC Canada

Fig. 5. Contours of real equilibrium fishing mortality $\tilde{F}_{eq}$ relative to F_{MSY} as a function of true and estimated natural mortality when the target harvest rate is (*a*) F_{MSY} and (*b*) $F_{35\%}$.

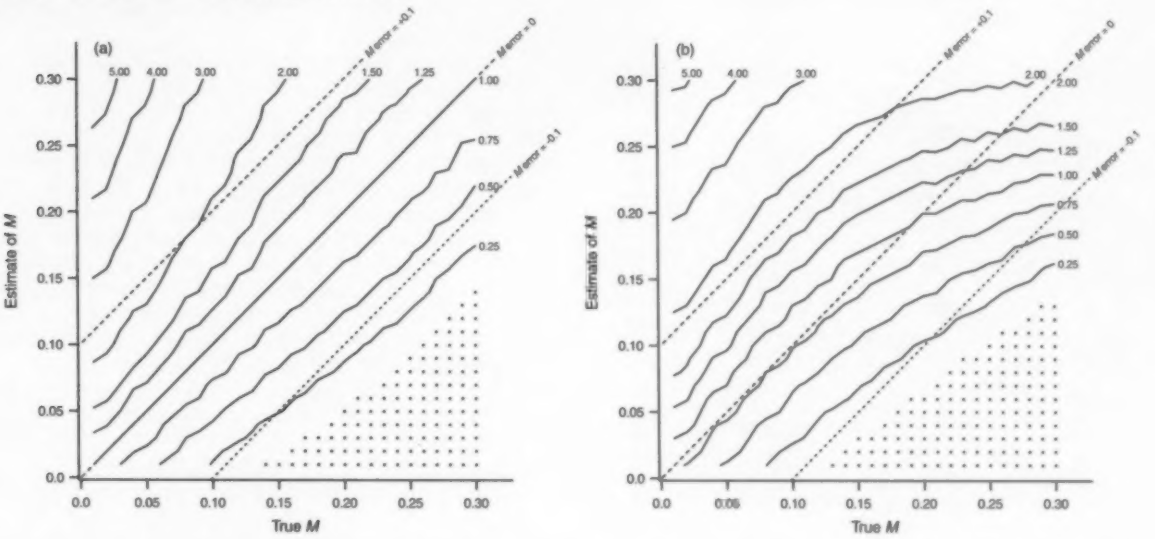
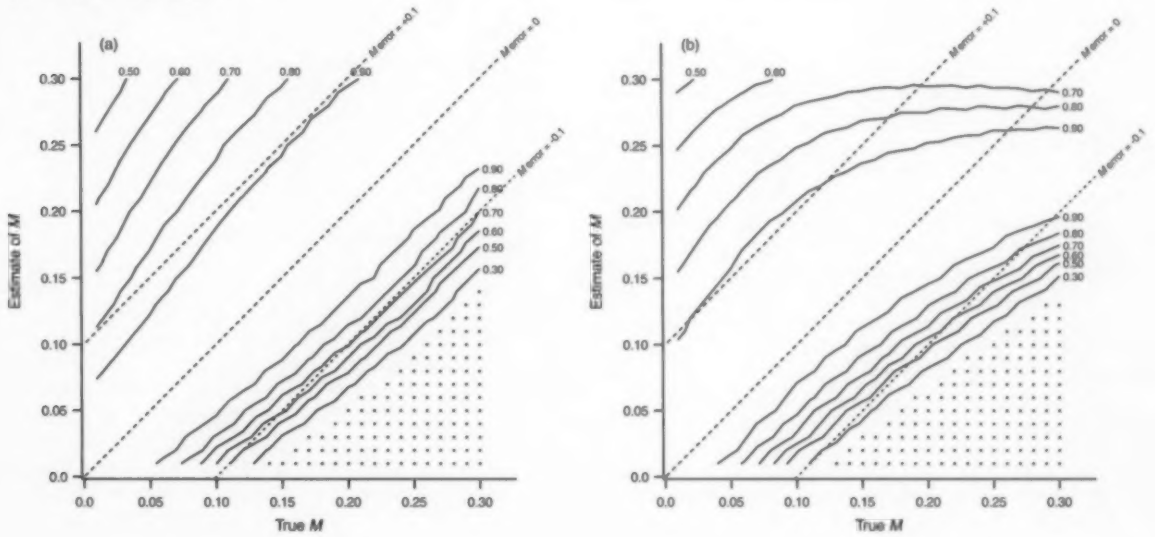


Fig. 6. Contours of equilibrium yield relative to MSY as a function of true and estimated natural mortality when the target harvest rate is (*a*) F_{MSY} and (*b*) $F_{35\%}$.



per effort is left out of the fit. After normalization, $(\hat{t}_a/t_a) = (\hat{s}_a/s_a)$, so an error in natural mortality will alter the survey selectivities proportionally just the same as the commercial selectivities and affect the stock size estimates and yield calculations in exactly the same way, so all of the analysis carries over to this case.

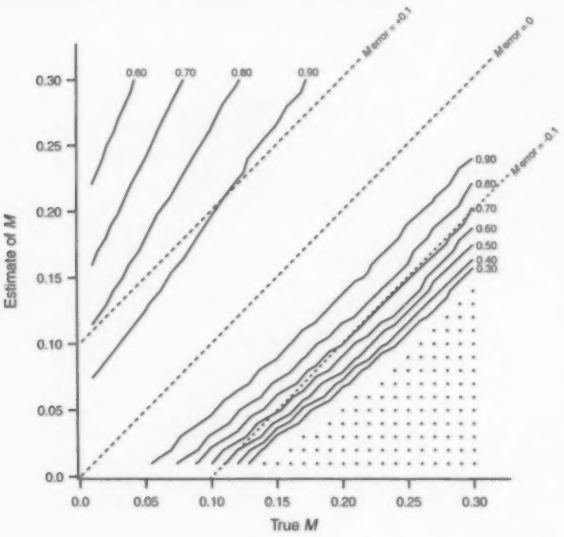
Note that when there is an error in $\hat{M}$, the estimated age-specific survey selectivities $\{\hat{t}_a\}$ that are required to reproduce the data depend on the age-specific commercial selectivities and the level of fishing mortality. If the estimated survey selectivities are viewed as a solution to the problem of reconciling the data with the wrong value of $\hat{M}$, then the solution is only good for a specific set of age-specific commercial selectivities and a specific harvest rate.

Nonseparable models

In cases where the separable model of fishing mortality is not used, the catch-at-age estimates are treated as known removals and year-class sizes are estimated directly along with the selectivities of the survey index to which the model is fitted. If the separable model is not correct, and the natural mortality rate is wrong, a good fit of the nonseparable model to deterministic data will not be possible. There will be large, systematic errors in the predicted survey index, simi-

© 1999 NRC Canada

Fig. 7. Contours of estimated MSY relative to true MSY as a function of true and estimated natural mortality.



lar to the bad fits of the (correct) separable model to data containing a strong trend or contrast in fishing mortality. This is because, as explained above, the estimated survey selectivities that reconcile the data and an incorrect value of $\hat{M}$ are only good for a single set of commercial selectivities and a single level of fishing mortality.

As explained above, good fits of the separable model are possible for a range of natural mortality rates even when fishing mortality is variable, so long as there is no marked trend or contrast, and the same is presumably true for commercial selectivity. That is, good fits of an age-structured model (separable or nonseparable) could presumably be achieved with a range of natural mortality rates even if commercial selectivity varies from year to year, so long as there is no marked trend or contrast in the time series. Where that is the case, the results obtained above should apply even when a nonseparable model is fitted.

Discussion

This paper has attempted to work out the effects of an erroneous natural mortality rate on each step of a simple stock assessment in order to find out what the eventual consequences are for the stock and to see whether there is any good strategy for dealing with uncertainty in the natural mortality rate, which is present in almost every assessment. A number of conclusions have emerged. Strictly speaking, these conclusions apply only to cases where the separable model is correct and there is not a strong trend in (true) fishing mortality, but as a practical matter, they should hold approximately in any case where an equally good fit can be obtained with a range of natural mortality rates.

Choosing a good external estimate of natural mortality

The results show that if the average (true) fishing mortality rate in the catch-at-age data is high enough (say 0.3 or greater), error in the natural mortality rate up to ±0.10 is not a serious concern. The working value can be carried through all the calculations and the recommended yields will be reasonable even though the stock size and harvest rate estimates may be poor.

If the average fishing mortality rate in the historical data is low, a modest overestimate of the natural mortality rate can produce large errors in stock size estimates, and the corresponding quota recommendations will represent very high real harvest rates in the short term. Underestimates of the natural mortality rate carry little penalty in terms of long-term yield and result in recommended real harvest rates lower than the nominal target. For management purposes, therefore, it makes sense to use a conservative (low) estimate rather than a point estimate of natural mortality as the working value when fishing mortality is low, or may be low. Nor is there any yield penalty in using a conservative estimate when fishing mortality is substantial, so this seems to be a sensible policy in general. The only hazard to avoid is a gross underestimate of natural mortality, which in the long term can result in a large underestimate of fishing mortality, a large overestimate of actual fishing mortality, and a very low real harvest rate (far below target).

Modeling and estimating selectivities

The selectivities of age groups that are mostly or fully recruited are quite well determined by the data even when $\hat{M}$ is wrong, so long as it not too close to Z. This means that it should always be possible to fit them individually rather than using a parametric function, and their behavior can be a useful diagnostic of a poor choice of $\hat{M}$. Moreover, while the use of a parametric function can provide a modest variance reduction if it is correctly specified, it can also result in large errors if it is incorrectly specified (Kimura 1990) or, more subtly, even if it is correctly specified but $\hat{M}$ is wrong.

Choosing a target harvest rate

Yield calculations done to choose a target harvest rate are affected by an error in $\hat{M}$ in several ways. For the case considered here, the estimate of F_{MSY} was less sensitive to both the true and the working values of natural mortality than was $F_{35\%}$. This is a good reason to attempt to use available spawner–recruit data to choose a harvest rate if there are enough points at low stock sizes to provide a meaningful estimate of the slope parameter. In terms of long-term yield, the estimated F_{MSY} and $F_{35\%}$ harvest rates both performed well when there was an error in $\hat{M}$.

Estimating natural mortality internally

It has been conjectured (e.g., Thompson 1994 and references cited therein) that the rate of natural mortality can be estimated internally so long as the selectivities are asymptotic. This paper has shown that for some common models with asymptotic selectivities, the conjecture is false when fishing mortality is not highly variable and the selectivities are estimated individually.

On the other hand, if it is the parameters of a selectivity function that are estimated (rather than individual selectivities), it is possible to estimate the natural mortality rate internally with the model studied here even when fishing mortality is constant, and with deterministic data the estimate is of course correct if the function is correctly spec-

© 1999 NRC Canada

ified. Sets of individually estimated selectivities can be located that reproduce the data exactly for any value of $\hat{M}$, and they are all very similar in shape, but only for the true value do the selectivities have *exactly* the shape required by the function.

The resulting estimate of the natural mortality rate is clearly artificial because it relies entirely on the precise shape of the specified selectivity function rather than on any information in the data. In practice, the form of the selectivity function is specified arbitrarily, and it is certainly not precisely the right one. Simulation exercises in which stochastic data are generated with a given selectivity function and then the natural mortality rate is estimated internally using knowledge of the form of the function (e.g., Schnute and Richards 1995) do not provide a real test of the performance or even the feasibility of internal estimates of the natural mortality rate in real assessments. This side effect of using a parametric selectivity function (i.e., making the natural mortality rate appear to be estimable when in fact it is not) is another reason to prefer individual selectivities.

In principle the rate of natural mortality can be estimated from deterministic data given sufficient contrast in fishing mortality and (or) selectivity, but in practice, real data usually contain too much noise and too little contrast for that. Given good fishing effort data $\{f_y\}$ and good estimates of total mortality, it should be possible to estimate the natural mortality rate by decomposing $Z_y = qf_y + M$ in the course of fitting the model, but even with some of the world's best data of both kinds, it was not possible to estimate the natural mortality rate of Pacific halibut by this method (McCaughran and Deriso 1988).

While estimating the natural mortality rate internally may not be practical in many cases, experimenting with different working values should be considered as a possible way to explain or eliminate systematic trends in the residuals, particularly around the index of relative abundance. If nothing else, the exercise will provide a broader view of the model fit than simply focusing on the results obtained with a single, more or less arbitrary working value.

Acknowledgements

Helpful comments on early versions of this paper were provided by Grant Thompson, Bruce Leaman, and especially Ana Parma, who suggested the closed-form solution of the selectivity equations and pointed out that all the long-term yield calculations were wrong.

References

Clark, W.G. 1991. Groundfish exploitation rates based on life history parameters. Can. J. Fish. Aquat. Sci. **48**: 734–750.

Collie, J.S., and Sissenwine, M.P. 1983. Estimating population size from relative abundance data measured with error. Can. J. Fish. Aquat. Sci. **40**: 1871–1879.

DeLury, D.B. 1947. On the estimation of biological populations. Biometrics, **3**: 145–167.

Deriso, R.B., Quinn, T.J., II, and Neal, P.R. 1985. Catch–age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. **42**: 815–824.

Doubleday, W.G. 1976. A least squares approach to analysing catch-at-age data. Int. Comm. Northwest Atl. Fish. Res. Bull. **12**: 69–81.

Fournier, D., and Archibald, C.P. 1982. A general theory for analyzing catch at age data. Can. J. Fish. Aquat. Sci. **39**: 1195–1207.

Kimura, D.K. 1990. Approaches to age-structured separable sequential population analysis. Can. J. Fish. Aquat. Sci. **47**: 2364–2374.

Leslie, P.H., and Davis, D.H.S. 1939. An attempt to determine the absolute number of rats on a given area. J. Anim. Ecol. **8**: 94–113.

McCaughran, D.A., and Deriso, R.B. 1988. Effect of biological assumptions on halibut production estimates. *In* Fishery science and management: objectives and limitations. *Edited by* W.S. Wooster. Lect. Notes Coastal Estuarine Stud. No. 28. Springer-Verlag, Berlin. pp. 5–29.

Mertz, G., and Myers, R.A. 1997. Influence of errors in natural mortality estimates in cohort analysis. Can. J. Fish. Aquat. Sci. **54**: 1608–1612.

Methot, R.D. 1989. Synthesis model: an adaptable framework for analysis of diverse stock assessment data. *In* Proceedings of the Symposium on Application of Stock Assessment Techniques to Gadids. *Edited by* L.-L. Low. Int. North Pac. Fish. Comm. Bull. No. 50. pp. 259–277.

Pope, J.G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Int. Comm. Northwest Atl. Fish. Res. Bull. **9**: 65–74.

Schnute, J.T., and Richards, L.J. 1995. The influence of error on population estimates from catch–age models. Can. J. Fish. Aquat. Sci. **52**: 2063–2077.

Thompson, G.G. 1994. Confounding of gear selectivity and the natural mortality rate in cases where the former is a monotone function of age. Can. J. Fish. Aquat. Sci. **51**: 2654–2664.

© 1999 NRC Canada

Effects of release parameters on recovery rates of tagged groundfish species

G.M. Fowler and W.T. Stobo

Abstract: A compilation of groundfish mark–recapture studies conducted in the Northwest Atlantic from 1953 to 1985 was examined by analysis of deviance to determine the effects of release parameters on subsequent recoveries of Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius virens*), and American plaice (*Hippoglossoides platessoides*). Fish length was a major determinant of recoveries for all four species, with recovery rates generally increasing with increasing fish length. Depth of capture was a significant determinant of recoveries for cod and haddock, with recovery rates decreasing as depth increased. Year of release was a significant determinant for all four species, with recovery rates declining in recent years. Declines in recovery rates of cod, haddock, and pollock occurred during certain months or seasons. Recovery rates decreased where fish were tagged in areas most distant from inhabited coastal regions, but the interpretation of this effect is complicated by changes in the fisheries over time between areas. Recovery rates also decreased with increasing catch size and varied between different tag types and capture methods. The results should prove useful in the design of tagging protocols and analysis of recovery data.

Résumé : Une compilation d'études de marquage–recapture de poissons de fond, effectuées dans l'Atlantique Nord-Ouest, entre 1953 et 1985, a été examinée par une analyse de la somme des carrés des écarts afin de déterminer les effets des paramètres de libération sur les récupérations subséquentes de morue franche (*Gadus morhua*), d'aiglefin (*Melanogrammus aeglefinus*), de goberge (*Pollachius virens*) et de plie (*Hippoglossoides platessoides*). Chez les quatre espèces, la longueur du poisson était un facteur déterminant important des récupérations, le taux de récupération augmentant généralement avec l'augmentation de la longueur du poisson. La profondeur de capture des poissons était un facteur déterminant significatif des récupérations dans le cas de la morue franche et de l'aiglefin, le taux de récupération diminuant avec l'augmentation de la profondeur. L'année de libération représentait un facteur déterminant chez les quatre espèces, le taux de récupération fléchissant ces dernières années. On a enregistré des baisses du taux de récupération chez la morue franche, l'aiglefin et la goberge au cours de certains mois ou certaines saisons. Le taux de récupération diminuait lorsque les poissons étaient marqués dans des régions très éloignées des régions côtières habitées, mais les modifications apportées à la pêche au fil du temps entre les régions compliquent l'interprétation de cet effet. Le taux de récupération a également diminué avec l'augmentation de la taille des prises et variait selon les différents types de marques et de méthodes de capture. Les résultats devraient servir à la conception de protocoles de marquage et à l'analyse des données sur les récupérations.

[Traduit par la Rédaction]

Introduction

Tagging studies have always been an important component of fisheries science, being used to describe fish movements, stock discreteness, and estimate abundance. But interpretation of the results of such studies is dependent on the recovery rate, which varies with differences between types of commercial fisheries, species, and tagging studies. Although legitimate concerns exist in the scientific community about the influence of fishing practices (e.g., location, gear, season) on recovery rates, limited investigation has occurred on features of the tagging studies themselves. Analyses among groundfish species have concentrated on differences between tags (McCracken 1957, 1959; Horsted 1963; Nielson 1963; Williams 1963; Wise 1963; Myhre 1966; Trumble et al. 1990; Fowler and Stobo 1991) or on differences in reporting tendencies between nations and fisheries (Poulsen 1957; Horsted 1963, 1965, 1967; Corlett 1967; Trumble et al. 1990; Thorsteinsson 1995). Less attention has been paid to such considerations as capture method (McCracken 1959; Wise 1963; Trumble et al. 1990; Thorsteinsson 1995), release location (McCracken 1957; Trumble et al. 1990; Thorsteinsson 1995), time of tagging (Wise 1963; Clay 1990), fish length (Wise 1963; Hoag 1975), and release group size (Hoag 1975).

A total of 203 767 groundfish of various species were tagged and released in the Northwest Atlantic during two separate programs conducted by the Canadian Department of Fisheries and Oceans covering the periods 1953–1973 and 1978–1985. Tagging activities were primarily concentrated throughout the Bay of Fundy, Scotian Shelf, and Gulf of St. Lawrence. The major species tagged were Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*),

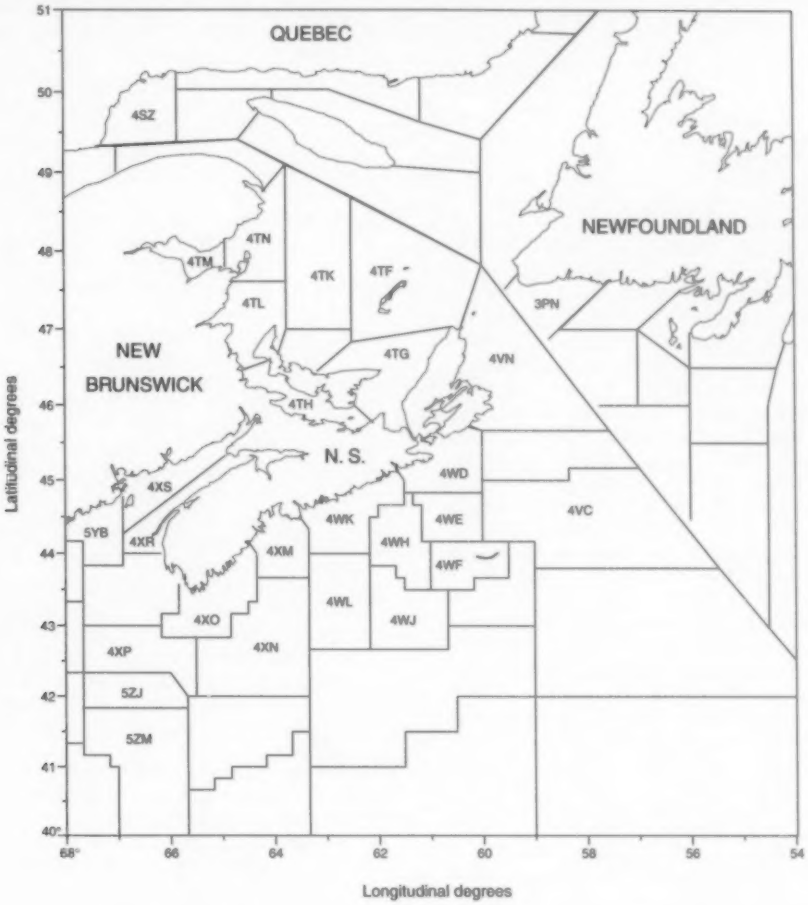
Received February 13, 1998. Accepted March 10, 1999. J14452

G.M. Fowler[1] and W.T. Stobo. Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada.

[1]Author to whom all correspondence should be addressed. e-mail: fowlerm@dfo-mpo.gc.ca

© 1999 NRC Canada

Fig. 1. NAFO unit areas where the tagging studies covered by this analysis were conducted.



pollock (*Pollachius virens*), and American plaice (*Hippoglossoides platessoides*), with 26 928 recoveries by the end of 1990. A wide variety of tagging methods, capture protocols, and environmental conditions were applied or encountered by the hundreds of tagging events represented by the two programs. These data provided an opportunity to examine the impacts of several features of the tagging event on subsequent likelihoods of tag recoveries, which should benefit the designs of future mark–recapture field protocols.

Features of the tagging event dealt with in this paper comprise tag type, fish length, year of release, release location, release group size (total number of fish tagged as a discrete event), month of release, depth of capture, capture method, and release program. The broad historical aspect of this analysis (release year and program), facilitated by the collation of many different tagging studies into a single database, may be unique in this field of interest. We have not found any treatment of the role of capture depth on tag recoveries in the literature. Most importantly, from the applied perspective of the tagger, the simultaneous consideration of multiple tag recovery factors over four different species provides a comparative reference point that can be used to gauge the actual relevance, as opposed to just the statistical significance, of the various potential effects.

Methods

Recovery rates (number of fish recovered per number of fish released) of tagged cod, haddock, pollock, and plaice were used to assess effects of the release environment on subsequent recoveries. We have included McCracken's (1957, 1959) data in this analysis. Only recoveries over 3 months post-release were considered, since biologists are usually concerned with seasonal and longer term movements of groundfish species, rather than immediate recaptures. The release numbers were adjusted by subtraction to correct for these disqualified returns. Given the exponential rate of decline of recoveries over time (Fowler and Stobo 1991), the partitioning of returns across several releases per species, and the low numbers of recoveries in recent years (93 fish in 1989 and 48 fish in 1990), we feel that our data adequately estimate total recoveries for all releases. Release areas are defined according to North Atlantic Fisheries Organization (NAFO) unit areas, as shown in Fig. 1.

Early exploratory analyses attempted to incorporate effects such as fishing mortality and effort estimates in combination with, or as substitutes for, year and broad-area effects for the 1978–1985 portion of our data. The available fisheries data proved too general-

© 1999 NRC Canada

ized to be useful in this study (whole-year Fs applied to a multi-area stock), where a particular month or area can be critical.

Several extreme recovery rates of 0.0 or 1.0 at the tails of our data distributions (less common lengths) were eliminated by requiring that all cases (i.e., all lengths within a tagged group of fish) have at least five fish released. The data set for cod was further adjusted to 10 fish released per case, not to handle extreme values but to reduce it to a manageable size to resolve computer memory limits. At time of release, cod, haddock, plaice, and much of the pollock were individually measured, while for some of the pollock releases, the untagged portion of the catch was subsampled to provide length frequencies. The unmeasured pollock were virtually all juvenile fish in the range of 18–23 cm. In our analyses, these unmeasured fish were assigned the median length from the subsampled length frequencies (20 cm). We felt that the margin of error over such a narrow length range was irrelevant to the analysis.

We use the term "T-bar" to identify tags that use a nylon filament with a T-shaped tip to anchor it in the muscle of the fish. The T-bar tag was used almost exclusively in the 1978–1985 program. This tag type has also been referred to as a spaghetti tag, an anchor tag, and a dart tag, all of which have progenitors in earlier tagging studies that are still in use today. As our own study includes the original spaghetti tag (used in the 1953–1973 program), we refer to its more recent derivative by the T-shaped nylon anchor. The unqualified Petersen disc tag in this paper refers to the most common version applied throughout the tagging studies covered, that being English-labelled with two yellow faces (discs). The rest of the Petersen disc tags in this study are identical to each other except for changes in labelling, colouring of the faces, or addition of a streamer (flag), as indicated by their names.

Analyses of deviance using SPLUS 3.3 (Chambers and Hastie 1993) were conducted to determine the relative magnitudes of influence of release parameters and predicted recovery rates generated to depict the nature of major effects. Analysis of deviance (a form of generalized linear modelling) is a derivation of analysis of variance, with some added versatility in the handling of distributions. We used it to distinguish numbers released and recovered for the purpose of weighting cases during binomial modelling (logit link). Tests of significance, degrees of freedom, and explained and residual model components are interpreted as for analysis of variance. All four species were initially examined using a two-way step function (Venables and Ripley 1994) to determine the order of entry of main effects. As the SPLUS step function may remove extremely insignificant terms from consideration, using a χ^2 test, stepwise addition of such eliminated terms was conducted manually to complete models (show insignificant effects) when necessary. The main effects models in each case were tested for under- or over-dispersion of responses relative to a perfect binomial distribution (dispersion = 1), for which only the plaice model tested negative. This test involved repeating the main effects model for each species using quasi-likelihood estimation of the dispersion parameter (McCullagh and Nelder 1987) to see if the significance of any of the effects changed as a result. The cod, haddock, and pollock models exhibited differences in significance of effects between the conventional and quasi-likelihood binomial analyses due to over-dispersion of recovery rates and so were rerun using the quasi-likelihood adjustment to account for the dispersion during modelling. This adjustment relaxes the distributional expectation to a more flexible assumption of symmetry. Any parameters not exhibiting significance at $\chi^2_{0.05}$ (perfect binomial model) or $F_{0.05}$ (quasi-likelihood binomial model) in the main effects models were removed prior to testing for interaction terms. Two-way interactions between main effects, whereby the nature of one main effect is modified nonadditively according to values of another main effect, were then modelled using the step function as for main effects. By preference, we would have produced a full all-effects-and-interactions model and eliminated terms using backward stepwise removal, but the number of parameters of such models would have required working memory resources beyond the capacity of our computers. The progression from a main effects model through interactions, with elimination of insignificant terms from further consideration at each order level (main effects first order, two-way interactions second order) was sufficient to allow for full forward/backward addition and removal of second-order terms for significant main effects. Interaction terms beyond second order (three-way or more interaction terms) were tested one at a time using forward entry. Insignificant interactions are not shown in our tables. The forms of the relationships (degrees of freedom) between recovery rates and continuous variables in the models were estimated subjectively with reference to scatterplots of recovery rates versus independent variates (Trellis plots were utilized where interactions were of concern). Various modelling procedures using general additive fitting or polynomial contraction/expansion with linear fitting were tried, but all resulted in at least one instance (per species) of model instability, and the stable models were characterized by far more interactions (and polynomial parameters) than we attained by visually estimating the nature of the curves. We believe that this can be attributed to the combined influences of several variables of mixed types in a very unbalanced design matrix.

Recovery rates for levels of a factor or values of a variate were estimated by prediction from the model. To derive a predicted response for a given effect, a single value was provided for each confounding term in a model (all other effects). We found predicted values more intuitive than parameter estimates and their interpretation less subject to confusion by interaction effects or unbalanced designs.

During discussion of the relative import of effects between models (species), we have made distinctions between major effects and statistically significant effects. The difference is subjective, with the designation of a major effect denoting that, in the opinion of the authors, the magnitude of the effect on recovery rates was large enough to warrant consideration in future tagging studies. Quantitatively, a major effect will generally represent over 10% of the explained deviance. An effect of lesser magnitude may not be regarded as a pragmatic concern, even though statistically significant.

Results and discussion

Overall release numbers, recovery numbers, and recovery rates are summarized in the Appendix. Total, 3 months post-release, and model data (minimum five or 10 releases per case) tallies illustrate changes in numbers and recovery rates between raw and filtered model data. These processing adjustments must be considered when comparing our results with previous studies, which have tended to use the total number of releases and recoveries. The detailed breakdowns by factor levels and variate values reflect model input data.

The highest unadjusted recovery rates were obtained for cod (20.1%) and the lowest for haddock (4.6%). Immediate recaptures (within 3 months post-release) accounted for about 15% (haddock) to 38% (pollock) of the total recoveries by species. After removing immediate recoveries and release cases of less than five haddock, pollock, or plaice or less than 10 cod per case, the resulting recovery rates by species were 20.7% (cod), 3.5% (haddock), 6.4% (pollock), and 9.5% (plaice). The extremely low returns for haddock may be due to successful targeting of spawners, such that the majority of fish tagged were physiologically weakened by re-

© 1999 NRC Canada

Table 1. Analysis of deviance of recovery rates for cod release parameters.

Quasi-likelihood model	df	Deviance explained	Residual df	Residual deviance	*F* mem	*P* (*F*) mem	*F* fm	*P* (*F*) fm	Dispersion
Null	na	na	1846	544.712	na	na	na	na	0.295
+poly(DEPTH, 3)	3	40.796	1843	503.916	82.647	0.00000	98.546	0.00000	
+poly(MONTH, 3)	3	42.222	1840	461.694	85.536	0.00000	101.990	0.00000	
+poly(LENGTH, 3)	3	47.216	1837	414.478	95.653	0.00000	114.053	0.00000	
+poly(YEAR, 3)	3	25.080	1834	389.398	50.810	0.00000	60.584	0.00000	
+AREA	18	73.721	1816	315.677	24.892	0.00000	29.680	0.00000	
+GEAR	3	6.438	1813	309.239	13.043	0.00000	15.552	0.00000	
+GSIZE	1	3.647	1812	305.592	22.164	0.00000	26.428	0.00000	
+TAG	5	8.337	1807	297.255	10.134	0.00000	12.084	0.00000	
+PROG	1	0.103	1806	297.152	0.626	0.42903	na	na	
–PROG									
+poly(YEAR, 3):AREA	14	35.910	1793	261.345	na	na	18.588	0.00000	
+poly(LENGTH, 3):poly(YEAR, 3)	9	7.427	1784	253.918	na	na	5.980	0.00000	
+poly(DEPTH, 3:poly(YEAR, 3)	9	4.898	1775	249.019	na	na	3.944	0.00005	
+poly(MONTH, 3):GSIZE	3	2.410	1772	246.610	na	na	5.821	0.00059	
+poly(LENGTH, 3):GSIZE	3	2.126	1769	244.484	na	na	5.136	0.00154	
+AREA:GEAR	1	0.513	1768	243.970	na	na	3.719	0.05395	0.138

Note: Where main effects are removed from a quasi-likelihood model due to insignificance, both full model (fm) *F*s and main effects model (mem) *F*s are presented. Removal of main effects from a model is depicted by a minus sign and italics.

Table 2. Analysis of deviance of recovery rates for haddock release parameters.

Quasi-likelihood model	df	Deviance explained	Residual df	Residual deviance	*F* fm	*P* (*F*) fm	Dispersion
Null	na	na	1090	150.489	na	na	0.138
+poly(LENGTH, 3)	3	17.055	1087	133.434	149.690	0.00000	
+poly(YEAR, 2)	2	38.213	1085	95.222	503.090	0.00000	
+TAG	3	29.599	1082	65.623	259.793	0.00000	
+poly(DEPTH, 2)	2	4.906	1080	60.716	64.594	0.00000	
+poly(MONTH, 2)	2	7.192	1078	53.524	94.685	0.00000	
+poly(GSIZE, 2)	2	1.462	1076	52.062	19.253	0.00000	
+AREA	11	6.415	1065	45.647	15.356	0.00000	
+poly(MONTH, 2):AREA	7	1.552	1058	44.095	5.837	0.00000	
+poly(LENGTH, 3):poly(YEAR, 2)	6	1.641	1052	42.454	7.201	0.00000	
+poly(YEAR, 2):poly(GSIZE, 2)	4	1.064	1048	41.390	7.005	0.00001	
+poly(LENGTH, 3):poly(GSIZE, 2)	6	0.648	1042	40.742	2.845	0.00942	
+poly(DEPTH, 2):AREA	17	1.815	1025	38.927	2.811	0.00012	0.038

Note: Where main effects are removed from a quasi-likelihood model due to insignificance, both full model (fm) *F*s and main effects model (mem) *F*s are presented. Note any possible effect of release program aliased by tag type.

productive activity. Alternatively, haddock may be generally less resilient than the other species in this study.

Analyses of deviance are depicted in Tables 1 (cod), 2 (haddock), 3 (pollock), and 4 (plaice). Model fits in terms of percent explained deviance were highest for pollock (94.8%), with intermediate values for haddock (74.1%) and plaice (68.4%), and with the lowest value for cod (55.2%). Plots of model residuals against an ideal normal distribution (QQ plots) are shown in Fig. 2. The cod model fits well, presenting no serious skews or anomalies. The distributions of the haddock and pollock data were kurtose with long tails, but symmetric. By contrast, the plaice data was skewed toward smaller values (one long tail to the right for larger values). The asymmetry in the fit for the plaice data could have distorted the final model, especially as regards effects explaining smaller amounts of deviance.

Undocumented variations in tagging protocols might account for the lesser explanatory power of the cod model relative to the other species in this analysis; about half the cod tagging was directed at spawning components, with no transcription of this type of data to clearly distinguish spawners from nonspawners. This may have introduced the confounding effect of a condition factor into the cod model, while tagging of the other species was performed under more consistent objectives. Statistically significant interactions occur in all models, but only the interaction between release year and release area for cod might be considered a serious component by magnitude of effect.

Length at release

Fish length was a major determinant of recovery rates for every species. It was the most important effect on pollock recoveries, explaining 70.7% of the total deviance in the model, and a major effect for plaice (13.1%), haddock

© 1999 NRC Canada

Table 3. Analysis of deviance of recovery rates for pollock release parameters.

Quasi-likelihood model	df	Deviance explained	Residual df	Residual deviance	*F* mem	*P* (*F*) mem	*F* fm	*P* (*F*) fm	Dispersion
Null	na	na	362	288.218	na	na	na	na	0.796
+poly(LENGTH, 2)	2	203.872	360	84.347	1263.078	0.00000	2192.098	0.00000	
+poly(YEAR, 2)	2	11.548	358	72.799	71.543	0.00000	124.164	0.00000	
+AREA	5	29.177	353	43.622	72.306	0.00000	125.488	0.00000	
+poly(GSIZE, 2)	2	12.098	351	31.524	74.955	0.00000	130.085	0.00000	
+poly(MONTH, 2)	2	2.589	349	28.935	16.039	0.00000	27.836	0.00000	
+GEAR	2	1.002	347	27.933	6.206	0.00225	10.770	0.00003	
+DEPTH	1	0.005	346	27.928	0.064	0.80023	na	na	
+TAG	1	0.084	345	27.844	1.045	0.30745	na	na	
–DEPTH–TAG									
+poly(LENGTH, 2):AREA	8	8.087	339	19.846	na	na	21.74	0.00000	
+AREA:poly(MONTH, 2)	7	2.567	332	17.279	na	na	7.885	0.00000	
+poly(LENGTH, 2): poly (MONTH, 2)	4	0.991	328	16.288	na	na	5.329	0.00036	
+poly(YEAR, 2): poly (MONTH, 2)	4	1.221	324	15.067	na	na	6.566	0.00004	0.047

Note: Where main effects are removed from a quasi-likelihood model due to insignificance, both full model (fm) *F*s and main effects model (mem) *F*s are presented. Removal of main effects from a model is depicted by a minus sign and italics.

Table 4. Analysis of deviance of recovery rates for plaice release parameters.

Binomial model	df	Deviance explained	Residual df	Residual deviance	*P* (χ^2)	Dispersion
Null	na	na	581	1704.143	na	3.848
+YEAR	1	636.327	580	1067.816	0.00000	
+poly(LENGTH, 2)	2	223.535	578	844.282	0.00000	
+AREA	9	168.978	569	675.304	0.00000	
+poly(GSIZE, 2)	2	34.300	567	641.004	0.00000	
+TAG	3	38.334	564	602.669	0.00000	
+poly(MONTH, 2)	2	5.160	562	597.509	0.07576	
+poly(DEPTH, 2)	2	1.933	560	595.576	0.38042	
–poly(MONTH, 2)–poly(DEPTH, 2)						
+YEAR:AREA	4	30.195	560	572.474	0.00000	
+YEAR:TAG	2	14.457	558	558.018	0.00073	
+poly(GSIZE, 2):TAG	6	18.752	552	539.266	0.00460	na

Note: Where main effects are removed from a quasi-likelihood model due to insignificance, both full model (fm) *F*s and main effects model (mem) *F*s are presented. Removal of main effects from a model is depicted by a minus sign and italics. Note any possible effect of release program aligned by tag type and release area.

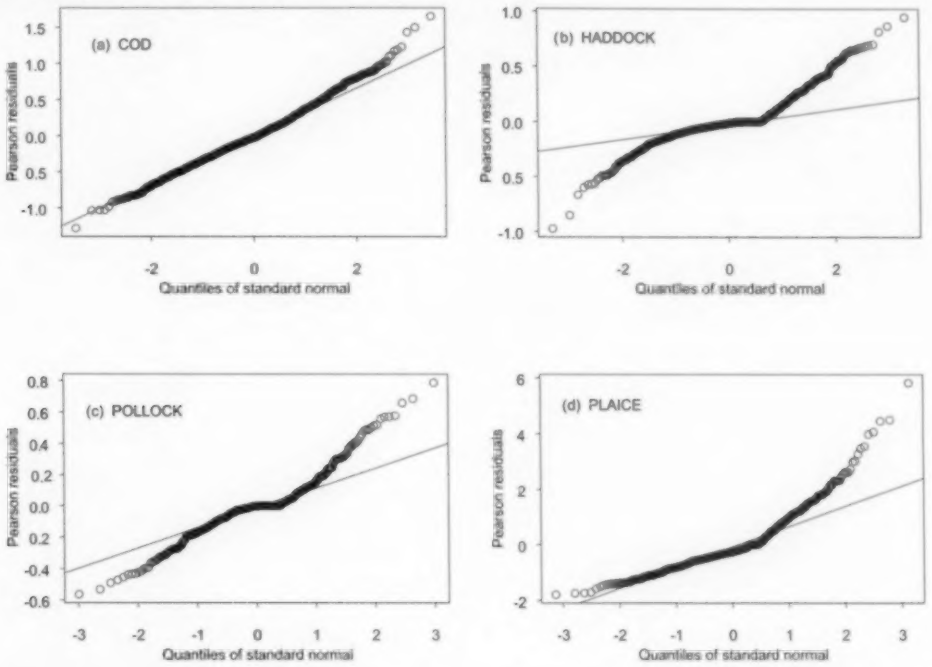
(11.3%), and cod (8.7%). For all species, the models predict increasing recovery rates with increasing length at release, which then either peak or plateau at some intermediate length and subsequently decrease at greater lengths (Fig. 3). Extremely low recovery rates are predicted for pollock and plaice under 20 cm. Pollock is an extreme case in which mass tagging of juvenile (harbour) pollock was conducted deliberately in coastal nursery areas. Although the smaller sizes of these fish may increase their vulnerability to tagging mortality, natural mortality preceding recruitment to the commercial fishery was probably a major contribution to the low recovery rates.

The initial positively sloped phase of the relationship between length and recovery rate has been reported before. Hoag (1975) found the same relationship for Pacific halibut (*Hippoglossus stenolepis*), and Wise (1963) noted low recoveries for cod under 40 cm. Beverton and Bedford (1963), looking at cod returns in relation to subjective condition indices (appearance and activity), found larger fish to be less affected than smaller fish with identical condition indices.

Our observation that recovery rates tend to plateau after reaching an intermediate length has not been previously reported, but it seems reasonable to expect fish greater than a certain size to be similarly tolerant of the tagging procedure. The subsequent decline in recovery rates for larger fish, however, is curious. This decline could be due to (*i*) senescence causing an increase in the natural mortality rate beyond some threshold length (age), (*ii*) increased tag loss with growth or time, (*iii*) lowered catchability through escapement (gear avoidance) of bigger fish, or (*iv*) lowered catchability of bigger fish due to distributional separation by area or depth from the bulk of the fished population (favour less fished or fishable habitats or locations). Reduced catchability and tag loss are the most likely explanations for the declining recovery rates observed for cod and haddock. An increase in natural mortality with age should have re-

© 1999 NRC Canada

Fig. 2. Quantile plots of the residuals from the final models for (*a*) cod, (*b*) haddock, (*c*) pollock, and (*d*) plaice. The straight line in each plot represents the perfect fit to a binomial model.



sulted in a narrower affected length range than that observed for these two species. Decline in recovery rate for larger specimens of plaice over a narrow length range may support the senescence scenario for flatfish, although sexual dimorphism (female plaice grow larger and older than males) could distort this picture if age-based distributional shifts occur. This interpretation implies that even large flatfish cannot develop the necessary swimming speeds to lower catchability by gear avoidance; towed bottom trawls are the dominant fishing gear for plaice.

Year of release

Year of release was a significant component in the models for all four species. It accounted for 37.3 and 25.4% of the deviance for plaice and haddock recoveries, respectively, with a lesser effect for cod and pollock. The magnitude of the effect of release year for cod was confounded by the interaction with release area. Recovery rates from tagging studies in the 1950s and early 1960s generally surpassed those of recent studies for all species (Fig. 4), although the sparsity of tagging data from the late 1960s and early 1970s made it difficult to generate continuous predictions across the entire time series.

The general decline in recovery rates over the years for every species probably mirrors the technological and managerial evolution of fisheries. Progressive change in emphasis from longlining to otter trawling over the period would have reduced the amount of individual handling of fish and hence reduced the probability of visually discovering tags. Increasing automation in catch processing methods, both at sea and in fish plants, likely reduced the tag reporting efficacy for the same reason. The willingness to report tag recoveries may have also declined due to increased restrictions on catch levels and fishing areas.

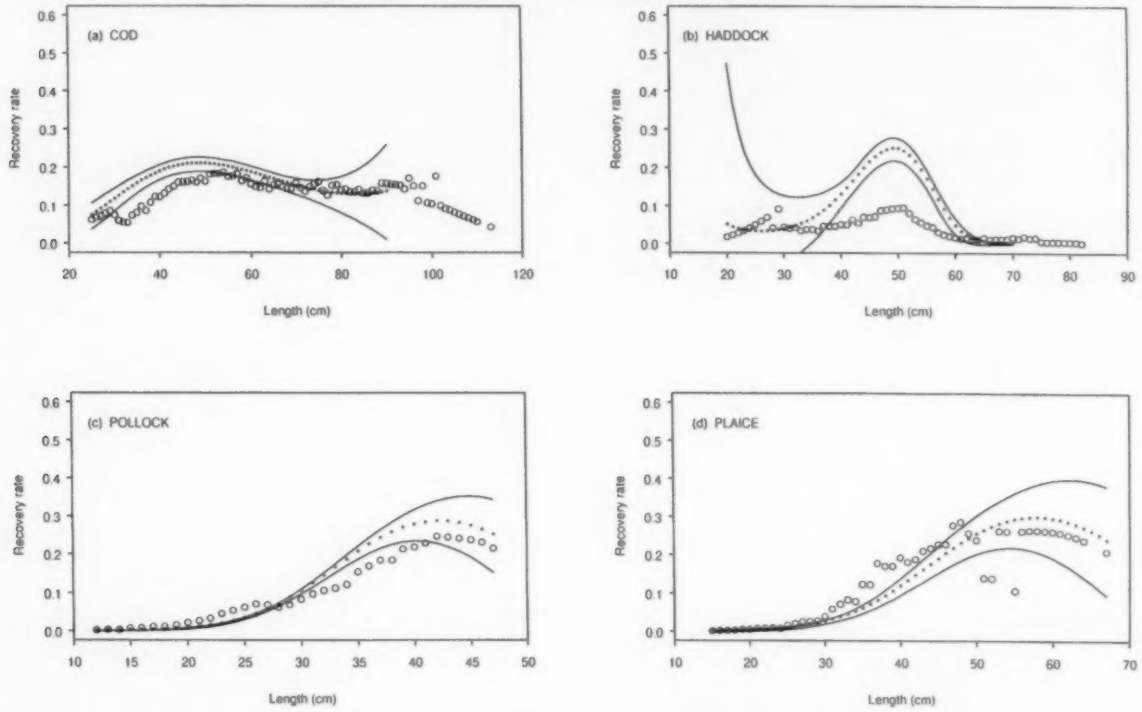
Area of release

Release area explained 13.5, 10.1, 9.9, and 4.3% of the deviance for cod, pollock, plaice, and haddock, respectively. It is not easy to explain the reasons for this effect due to the difficulty in ascribing differences in recovery rates to differences in fisheries between areas, especially over years. The importance of the interaction for cod between year and area illustrates the problem. Predictions for cod (Fig. 5) indicate better recoveries for eastern Scotian Shelf releases (4VC, 4VN, 4WE) than releases from central Shelf areas (4WH, 4WK, 4XM), the southern Gulf of St. Lawrence (4TG, 4TL), or the Bay of Fundy (4XS). This difference could be variously associated with aspects of the fisheries that we have not been able to consider (e.g., Danish seining, common only on the eastern Scotian Shelf; an anomalously high representation of recovery submissions from St. Pierre and Miquelon vessels fishing in 4V; differences in fishing effort). The difference could also be due to the deliberate tagging of spawning and post-spawning fish, an aspect of the tagging environment (fish condition factor) for which we have no data to include in the analysis. About half of the tagging studies on cod included in this analysis were directed at spawning fish, but no consistent records were taken of the protocols followed or of individual condition status on release.

With pollock, Scotian Shelf (4W, 4XO) releases gave generally better predicted recoveries than releases in the Bay of

© 1999 NRC Canada

Fig. 3. Mean fitted (circles) and predicted (asterisks) recovery rates with standard errors of predicted values (solid lines) against fish length at release for (*a*) cod with a prediction for 250 fish otter trawled from 92 m in NAFO area 4VC during May 1979 and tagged with T-bars, (*b*) haddock with a prediction for 150 fish from 92 m in NAFO area 4XS during November 1957 and tagged with Petersen discs, (*c*) pollock with a prediction for 3000 fish caught in a cod trap in NAFO area 4WD during May 1981, and (*d*) plaice with a prediction for 100 fish taken in NAFO area 4TG during 1979 and tagged with Petersen discs.



Fundy (4XR, 4XS) or southwestern Newfoundland (3PN). The high pollock recovery rates in 4XO appear explicable, since many of the recoveries came from recreational anglers rather than commercial fishers. The personal attention devoted to fish caught in this fashion would certainly enhance the likelihood of noticing a tag, while the probable novelty of the experience would enhance the likelihood of submitting the tag for a reward. The reasons for the high pollock recovery rates in 4W are difficult to explain due to fisheries and other biological effects, similar to the situation for cod.

For plaice, Scotian Shelf (4W, 4X) tagging studies proved too unique with respect to tag types, years of release, and release group sizes to allow comparisons. Within the Gulf of St. Lawrence (4T), recovery rates appeared to improve along a rough gradient from southeastern to northwestern areas. This may correspond to movements of plaice, assuming greater transiency in southeast waters and more prolonged residency in northeast waters. It may also show an affiliation between higher recovery rates and settled coastal regions, since historical choice of settlement locations could also reflect optimal fishing grounds, and thus fish distribution.

Several combinations of standard values for release parameters were used to try to generate predictions on release areas from the haddock model without success. We do note that the better recovery rates seem to be associated with the more nearshore areas, perhaps in correlation with the generally greater fishing effort of nearshore as opposed to offshore locations. Thorsteinsson (1995) also noted higher recovery rates for Icelandic cod from nearshore tagging than from offshore tagging.

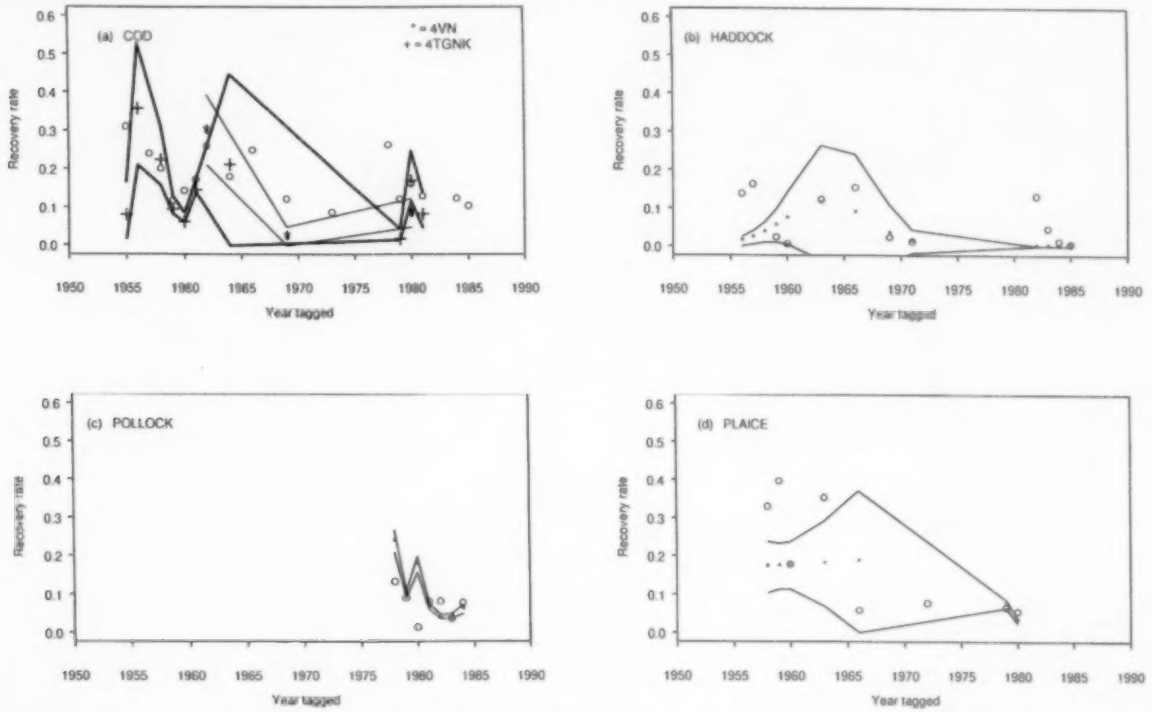
Tag type

Tag type was a significant effect in all but the pollock model. It was a major effect for haddock (explaining 19.7% of the deviance), while a relatively minor effect in the plaice (2.2%) and cod (1.5%) models. Higher recovery rates were predicted for haddock with red and white Petersen disc tags than with yellow Petersen disc tags (Fig. 6). Higher recovery rates were predicted for plaice with red and yellow Petersen disc tags than for plaice with dagger tags and for plaice with T-bar tags than for plaice with monocolour yellow Petersen disc tags. Higher recovery rates were predicted for cod with Petersen disc tags and Lea hydrostatic tags than for cod with T-bar tags, with the worst predicted recoveries for cod with spaghetti tags. Within Petersen disc tags of the same manufacture, predicted recovery rates for French-labelled tags exceeded those for English-labelled tags, while English-labelled tags in turn gave higher predicted recovery rates than bilingual tags.

In a general overview of tag effects, it appears that the currently popular T-bar tag is similar to the previously common Petersen disc tag with respect to recovery rates. The

© 1999 NRC Canada

Fig. 4. Mean fitted (circles) and predicted (asterisks) recovery rates with standard errors of predicted values (solid lines) against year tagged for (*a*) cod with two predictions for one hundred 50-cm fish otter trawled from 46 m in NAFO areas 4VN and 4TGNK during September and tagged with Petersen discs, (*b*) haddock with a prediction for one hundred and twenty-five 50-cm fish from 82 m in NAFO area 4XP during March and tagged with Petersen discs, (*c*) pollock with a prediction for two thousand 30-cm fish trapped (improvised gear) in NAFO area 4WD during May–June, and (*d*) plaice with a prediction for one hundred 35-cm fish taken in NAFO area 4TG and tagged with yellow and yellow–red Petersen discs.



models predict higher recovery rates for the T-bar tag than for the yellow Petersen disc tag on plaice, and conversely, higher recovery rates for the yellow Petersen disc tag than for the T-bar tag on cod. On the few occasions in which the normally yellow Petersen discs were replaced with discs having one red face and one white or yellow face (some haddock and plaice releases), higher recovery rates were predicted for the Petersen disc tag than for any other. This might imply an improved visual contrast of red over yellow (or white) against the colour of the fish. Anecdotal information suggests that red discs were rarely used due to concern that the red face might be visually mistaken for blood and not detected.

If the T-bar tag versus Petersen disc tag contrasts are reasonable, they still provide no indication of whether the differences are attributable to tag visibility, tag loss, or handling stress (mortality). T-bar tags are more rapidly applied (via tagging guns) than Petersen disc tags (hand-tied with wire). The dismal performance of hand-tied spaghetti tags relative to either Petersen disc or T-bar tags suggests increased handling stress as the explanation, but only if it is assumed that tag loss was not a problem. Failure to consistently tie a secure knot when applying spaghetti tags could have lowered potential recovery rates through tag loss.

The enhanced recoveries of French-labelled Petersen disc tags, infrequently applied to Gulf of St. Lawrence (4T) cod, over Petersen disc tags with bilingual or English labels may reflect a simple failure to communicate or just prevailing attitudes.

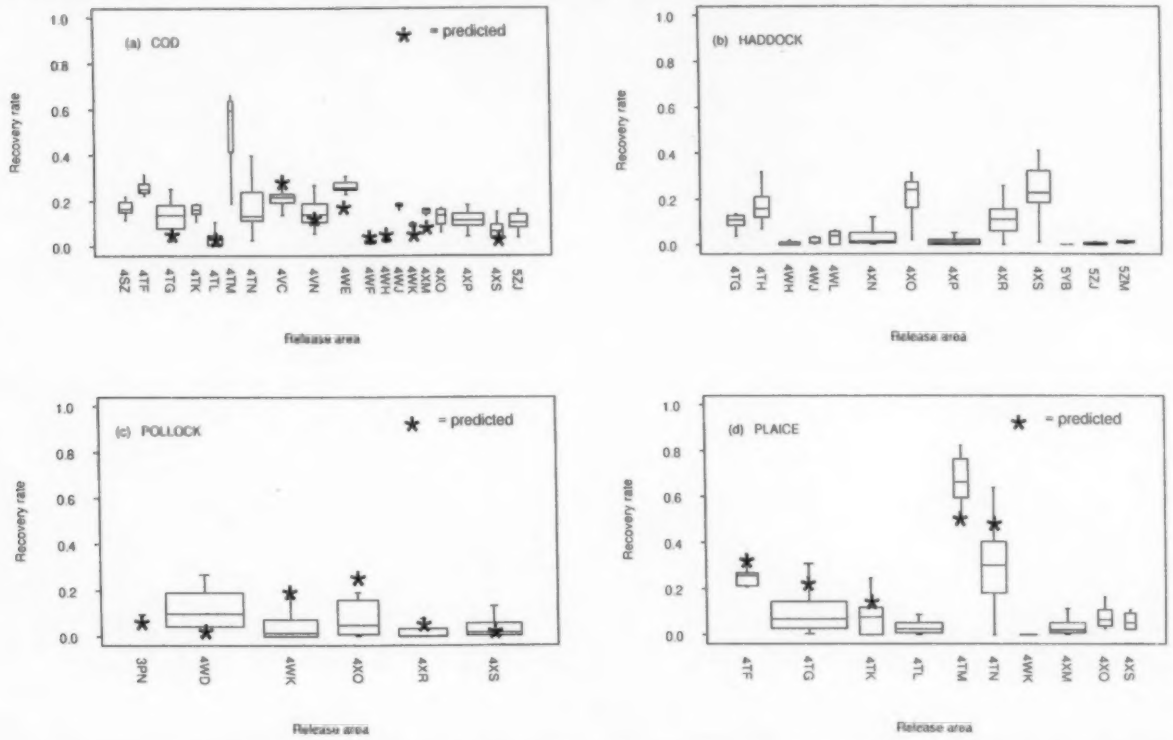
The two species for which T-bar and Petersen disc tags are compared in this study (cod and plaice) were also the subjects of an explicit comparison between tag types conducted in 1979 (Fowler and Stobo 1991), in which T-bar tags outperformed Petersen disc tags on cod and no difference was found between T-bar tags and Petersen disc tags on plaice. The contrast made in the current study between English-labelled T-bar and Petersen disc tags includes the same data used in the 1979 study, although they only represent a fraction of the total data in this analysis. Exploratory analyses of various subsets of the data were undertaken to discern why results differed between the two studies. The findings of both studies were confirmed, but no explanation for the difference between the Fowler and Stobo (1991) study and the current analysis could be found in the available data.

Month of release

Month of release was a significant effect for cod, haddock, and pollock, but not for plaice, and had a major impact only on cod recovery rates (explaining 7.8% of the deviance). Cod predictions (Fig. 7) depict a progressive de-

© 1999 NRC Canada

Fig. 5. Box-and-whisker plots of mean fitted recovery rates and predicted recovery rates for NAFO areas of release for (*a*) cod with a prediction for two hundred and fifty 55-cm fish otter trawled from 55 m during September 1979 and tagged with T-bars, (*b*) haddock, (*c*) pollock with a prediction for three thousand 20-cm fish trapped (improvised gear) during August 1979, and (*d*) plaice with a prediction for one hundred and fifty 36-cm fish taken during 1959 and tagged with yellow–red Petersen discs. The box encompasses the most central 50% of the recovery rates, the width of the box is proportional to number of cases in the data set, the vertical whiskers bound the data distribution excluding outliers, and the horizontal bar inside the box is the median recovery rate.



cline in recovery rates from January through April, followed by a plateau for the remainder of the year. Much of the post-March tagging may have been directed at spawning fish, such that many of them might have been weakened and perhaps more susceptible to capture stress mortality. With haddock, the poorest recovery rates were obtained from releases in late winter – early spring, but due to aliasing, we were unable to find a set of release parameter standard values to provide a prediction. As haddock spawn mostly around March, the majority might have been weakened. Recovery rates increased through the summer (regarding the July datum as anomalous) and fall release months.

Virtually no recoveries were obtained from late winter releases of pollock. Such an extreme result may reflect drastic mortality, possibly caused by damage to gill epithelia by freezing air temperatures, but we have no temperature data to substantiate this hypothesis. With the inclusion of month in three interaction terms for the model exceeding its contribution as a main effect, we did not attempt to decipher differences in the remaining months.

Depth caught

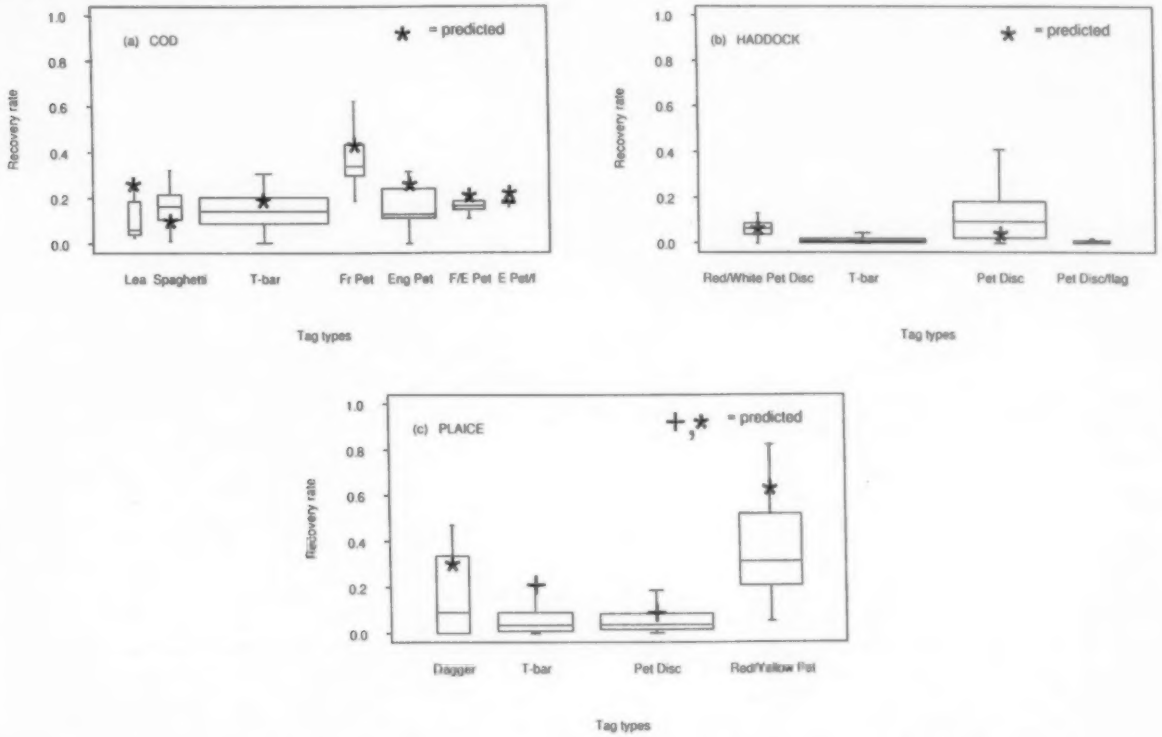
Depth of capture proved important only for cod (explaining 7.5% of the deviance), although it was also statistically significant (at 3.3% of the deviance) for haddock. A predominant trend of decreasing recovery rates with increasing depth of capture is evident for both species (Fig. 8), with the possibility of increasing recovery rates with increasing depth for the shoalest depths (0–55 m). No relationship is evident for plaice and all of the pollock were tagged in water less than 20 m deep, too narrow a range to exhibit any effect of a depth gradient.

Declines in recovery rate with cod and haddock initially caught in deep water are difficult to attribute to a specific cause. Depth serves as a proxy for duration of capture stress during haul-back, damage to gas bladders resulting from pressure differentials, and thermal shock during warmer months if fish are hauled through a thermocline. Very few cod or haddock were taken above the thermocline, so variation in this study should relate only to differences between depths below the thermocline (almost all fish undergoing thermal transition), suggesting that bladder damage and (or) stress duration would seem likelier causes. Considering the absence of an effect of capture depth on recovery rates for plaice, which have no swim bladder, bladder damage seems slightly more explanatory than stress duration.

The possible increase in recovery rate with increasing depth over the first 0–55 m of the depth profile we can only

© 1999 NRC Canada

Fig. 6. Box-and-whisker plots of mean fitted recovery rates and predicted recovery rates by tag type for (*a*) cod with a prediction for two hundred and fifty 55-cm fish longlined from 37 m in NAFO area 4TN during June 1955, (*b*) haddock with a prediction for one hundred and fifty 50-cm fish from 101 m in NAFO area 4XN during March 1957, (*c*) plaice with two predictions, one for two hundred and fifty 36-cm fish taken in NAFO area 4TG during 1979 (crosses) and one for one hundred and fifty 40-cm fish taken in NAFO area 4TN during 1959 (asterisks). The box encompasses the most central 50% of the recovery rates, the width of the box is proportional to number of cases in the data set, the vertical whiskers bound the data distribution excluding outliers, and the horizontal bar inside the box is the median recovery rate.



speculate might be related to an initial period of rapid respiration (panic response) overlapping the period of time exposed to the air during tagging. Perhaps an active fish is more likely to be injured by thrashing or gill damage (breathing air) than a fish that has been exhausted before reaching the surface.

Capture method

Capture method was a minor but statistically significant effect on cod recoveries (1.2% of the deviance) and pollock recoveries (0.4% of the deviance). All the plaice and haddock in our models were caught for tagging by otter trawl. A substantial proportion of cod, however, were caught using longlines, and these releases gave slightly better recoveries than trawled or handlined releases (Fig. 9). For pollock, the use of conventional cod traps gave better recovery rates than scaled-down traps improvised for research.

The higher recovery rates for cod caught by longlining than for cod caught by trawling seem intuitively reasonable, since the trawled fish would be expected to incur more injury from compaction in the net, impacts with the deck, and confinement in crowded saltwater tanks while waiting to be tagged. However, by the same reasoning, handlining should have given the best results of all the capture methods. The absence of this result may be due to sample size: the extrapolation to predict recovery rates for a release of 500 handlined fish to achieve a comparison with other capture methods was simply too much.

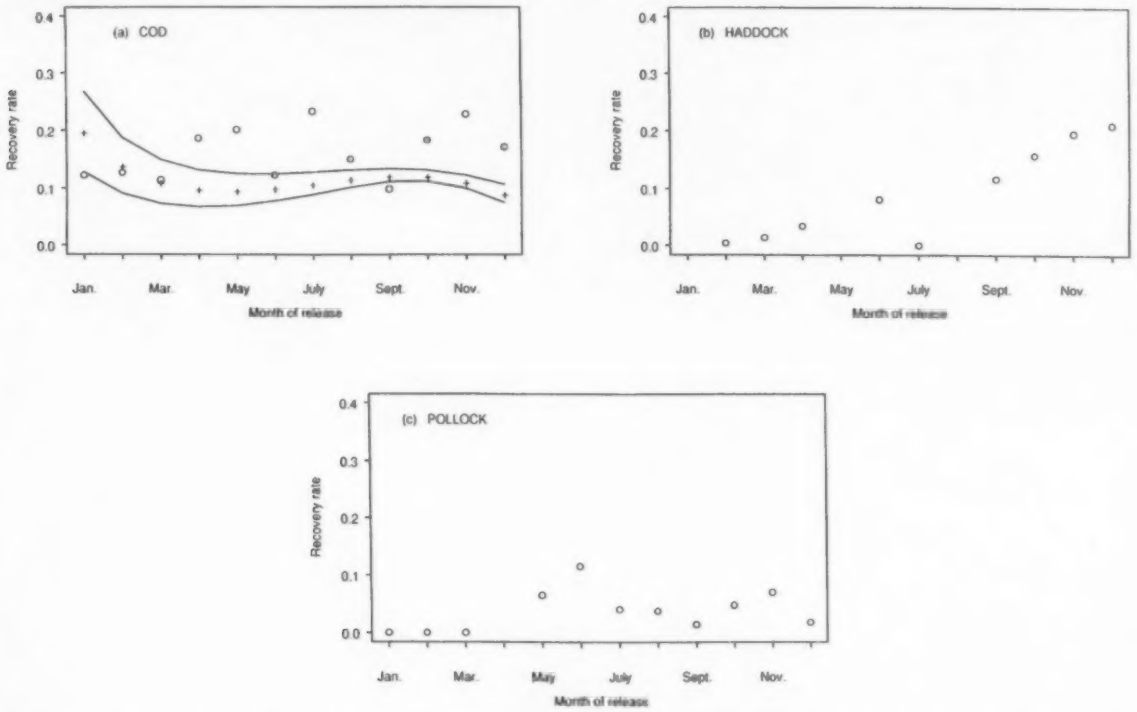
The cod trap was very rarely used to tag cod and could not be directly compared with other gears used to capture cod for tagging due to aliasing. And the only other use of the cod trap for tagging was for pollock, where it could only be compared with noncommercial traps that lacked any benefit of historical development to minimize damage to the fish.

Release group size

Release group size was statistically significant in every model (4.2% of the explained deviance for pollock, 2.0% for plaice, 1.0% for haddock, 0.7% for cod), although the magnitudes of effect were all quite small. A very slightly sloped increase in recovery rate with increasing release group size (Fig. 10) is suggested for pollock releases, decreasing recovery rates with increasing release group sizes were observed for cod tagging, and dome-shaped (increase–peak–decrease) recovery rates with increasing release group size were ob-

© 1999 NRC Canada

Fig. 7. Mean fitted (circles) and predicted (asterisks) recovery rates with standard errors of predicted values (solid lines) against month of release for (*a*) cod with a prediction for one thousand five hundred 55-cm fish otter trawled from 92 m in NAFO area 4VN during 1980 and tagged with T-bars, (*b*) haddock, and (*c*) pollock.



served for plaice. We failed to find a set of standard values of release parameters to generate a prediction for haddock due to aliasing, but note that the fitted values depict a pattern similar to that for plaice. This similarity could imply the same increase–peak–decrease in haddock recovery rate with release group size.

Hoag (1975) noted declining recovery rates with increasing catch size for Pacific halibut. Fish associated with small tagging releases are probably subject to less physical damage than those of large releases due to less compaction in the gear (if a net) and on deck (if dumped) as well as less physiological stress due to shorter periods of time in holding tanks waiting to be tagged. Such differences could account for the higher recovery rates observed for smaller releases of cod, haddock, and plaice. Thorough analysis of this effect on recovery rates would require information on the actual size of the catch (for which we have only number tagged as a surrogate), the number of tagging personnel involved, and the length of time between initial capture and tagging. Unfortunately, this information was not systematically documented during the field operations and (or) processing of the tag release data used in this study.

It was noted above that higher recovery rates were obtained for cod initially caught with longline gear than for cod caught with otter trawls. One possible explanation for the superiority of longline gears for cod might have to do with the actual catch sizes involved being smaller than indicated by the data. Our data contain instances of apparent release group sizes of over 1000 fish for otter trawlers and over 500 fish for longliners. Discussions with personnel involved in various tagging projects indicate that trawled catches of over 500 fish at a time were common, whereas longline gear sets rarely provided more than 100 fish. It is thus possible that some longline sets were combined during processing. Handlining would always have been an essentially one-to-one operation between tagger and fish, thus reducing the likelihood of physical damage due to compaction and minimizing physiological stress due to handling time. If actual set catch sizes had been reliably documented, it is possible that no differences between methods would have been detected.

Release program

The difference in recovery rates between the 1953–1973 and 1975–1985 tagging operations was not an important consideration in any model. Year of release adequately represented long-term differences in recovery rates for cod. Other main effects took precedence for haddock and plaice, aliasing release program out of those models.

Conclusions

To evaluate potential recovery rates for a specific tagging study, the nature of all possible effects in the four models should be considered to predict their relevance to the situation. Many of the effects that we examined can be interpreted independently of the species in a given model, and much of the variation in relative importance of effects be-

© 1999 NRC Canada

Fig. 8. Mean fitted (circles) and predicted (asterisks) recovery rates with standard errors of predicted values (solid lines) against depth of capture for (*a*) cod with a prediction for one thousand 40-cm fish otter trawled in NAFO area 4VC during September 1979 and tagged with Petersen discs and (*b*) haddock.

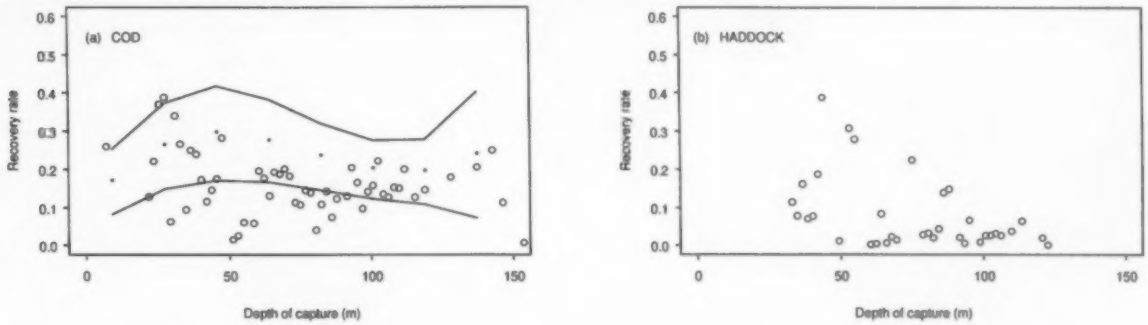
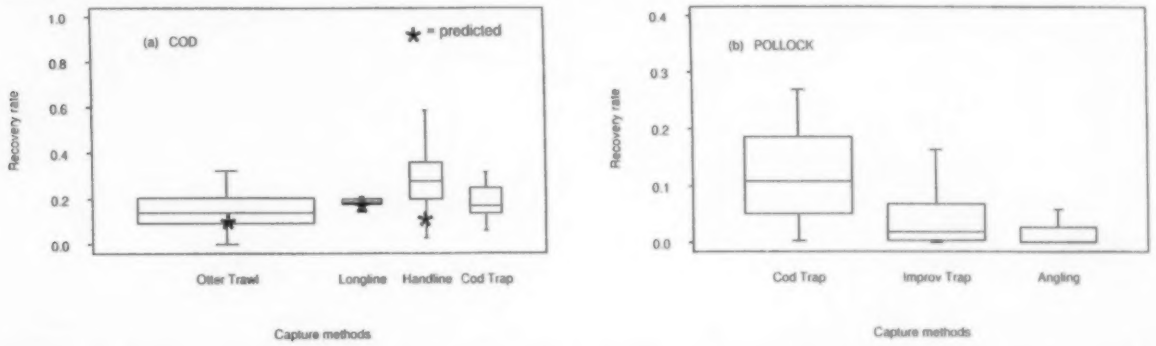


Fig. 9. Box-and-whisker plots of mean fitted recovery rates and predicted recovery rates by capture method for (*a*) cod with a prediction for five hundred 50-cm fish taken from 37 m in NAFO area 4VN during September 1980 and tagged with T-bars and (*b*) pollock. The box encompasses the most central 50% of the recovery rates, the width of the box is proportional to number of cases in the data set, the vertical whiskers bound the data distribution excluding outliers, and the horizontal bar inside the box is the median recovery rate.



tween models can be ascribed to differences in tagging objectives rather than to unique aspects of the species tagged. Some effects (e.g., month, location) can represent a combination of attributes that are unique to a given species (e.g., spawning, migration), while others are more generally applicable (e.g., temperature, fishing effort).

Of the eight effects that we found to be significant determinants of potential recovery rates, two of them (year, area) may not provide insights into use in future tagging studies. Year of release may represent historical change, over which an experimental designer has no control, and the geographic location of release is likely to be imposed by the objectives of the tagging program. However, the remaining six effects (month of release, tag type, fish length, capture depth, method of capture, release group size) are controllable, to some degree, within the usual constraints of a tagging program.

Month of release will almost always be a function of tagging objectives, although flexibility in timing or procedures could mitigate against early post-release mortality or recapture. Adjusting the timing of releases by a few days or weeks in some instances could avoid concentrated commercial fishing to minimize immediate recoveries. Constraining winter tagging programs to favour unseasonably warm days, or adoption of a technique similar to that of Clay (1990) whereby the fish are kept submerged during the tagging operation, might reduce the possibility of freezing temperatures as a potential source of mortality.

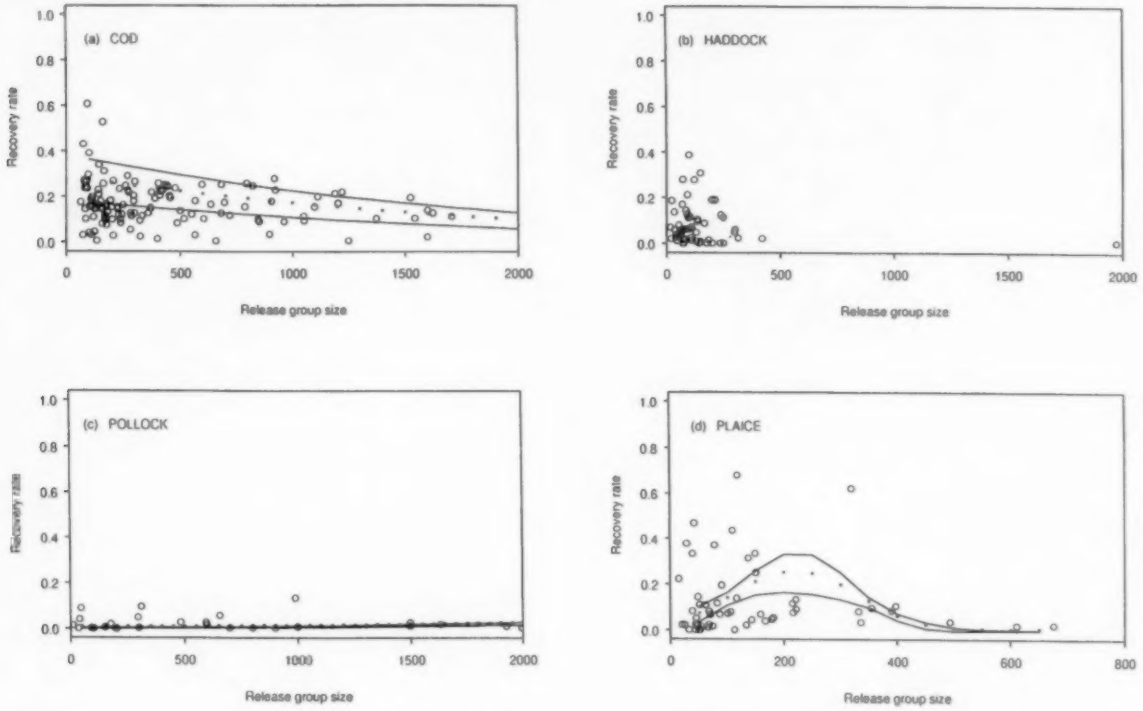
Most tags available today are probably quite adequate in terms of application speed and subsequent retention, but given the higher recovery rates that we found with red and white–yellow Petersen disc tags, some consideration of the unique visual contrast (color, pattern) between a tag and the targeted species might improve recoverability by fishery and processing personnel. The speed and ease of application of the T-bar tag might be considered an overriding factor in selecting it in preference to the Petersen disc tag.

Because small fish give the poorest recoveries and large fish also exhibit lower recoveries, intermediate sizes might be targeted if not problematic to the tagging protocol. If juveniles are being specifically targeted or the fish are not individually measured, then the tagging protocol will negate any application of size constraints to enhance recoveries. Otherwise, some form of size selection may be of benefit, especially if applying expensive tags. It becomes a question of whether it is worth the time and cost to tag fish smaller or larger than some optimal length range and conversely whether it is worth the trouble to exclude them.

The shallower the depth at initial capture, the better the recovery rate, but any depth less than 55 m will likely result

© 1999 NRC Canada

Fig. 10. Mean fitted (circles) and predicted (asterisks) recovery rates with standard errors of predicted values (solid lines) against release group size for (*a*) cod with a prediction for 50-cm fish otter trawled from 92 m in NAFO area 4VN during February 1980 and tagged with T-bars, (*b*) haddock, (*c*) pollock with a prediction for 20-cm fish cod-trapped in NAFO area 4WD during June 1979, and (*d*) plaice with a prediction for 36-cm fish taken in NAFO area 4TG during 1979 and tagged with T-bars. Note that the *x*-axis for the plaice plot only goes to 800, while the other three plots go to 2000.



in improved recovery rates, in the absence of a thermocline. If a thermocline exists, fish initially captured above it should provide good recovery rates. The requirements of a particular tagging program could certainly mitigate against depth of capture restrictions on some species, but in many situations the depth at which capture occurs relates to where fish are first found, without searching for shoaler concentrations. And to reduce possible damage due to thrashing, consideration might be given to delaying exposure of fish caught very close to the surface, so that the fish might be less active once out of the water.

Capture method, inclusive of catch size, can be controlled regardless of gear type. The key considerations would appear to be the number of fish caught per set and the processing speed. Improved recovery rates would probably occur by setting upper limits to the number of fish caught per set and the number of fish to be tagged per person per catch event via gear parameters (e.g., number of lines, size of net, duration of trawl).

Based on the various results of this study, we would suggest the following as general guidelines for tagging commercial groundfish species.

(*i*) Unless juveniles are crucial to the objectives of the experiment, fish under 20 cm should be avoided.

(*ii*) Where tagging of actively spawning fish is not a program objective, tagging should be scheduled to avoid peak spawning times.

(*iii*) Fish should not be tagged in air temperatures below freezing, unless the tagging operation can be designed to safeguard fish during processing (e.g., onboard tanks). This need not preclude winter months, provided the protocol is flexible enough to skip periods of time when freezing can occur.

(*iv*) Times and areas of heavy fishing activity should be avoided to reduce immediate (non-informative) recaptures.

(*v*) Where feasable, fish should be taken from shallow waters above any thermocline, especially if they have gas bladders.

(*vi*) Commercial fishing gear should be used in preference to ad hoc alternatives. These capture methods have evolved to minimize damage to the fish.

(*vii*) Restrictions should be included in capture protocols to keep the catch size down to about 150 fish per tag team per tagging event.

(*viii*) The visual contrast, in terms of colours and shapes, between the tags and the fish should be considered when selecting tags.

Acknowlegements

An analysis of this nature would not have been possible without the effort of innumerable research staff who participated in the tagging studies and even more people involved in the fisheries who returned the tags. We also thank Stratis

© 1999 NRC Canada

Gavaris, Tom Hurlbut, and three anonymous reviewers for providing constructive comments on previous drafts of this manuscript.

References

Beverton, R.J.H., and Bedford, B.C. 1963. The effect on the return rate of condition of fish when tagged. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 4. pp. 106–116.

Chambers, J.M., and Hastie, T.J. 1993. Statistical models in S. Chapman and Hall, London, U.K.

Clay, D. 1990. Tagging demersal marine fish in subzero temperatures along the Canadian Atlantic coast. Am. Fish. Soc. Symp. **7**: 147–151.

Corlett, J. 1967. Cod tagging off Labrador, November–December 1962. Int. Comm. Northwest Atl. Fish. Res. Doc. No. 67/54.

Fowler, G.M., and Stobo, W.T. 1991. Comparative recoveries of spaghetti tags and Petersen disc tags on Atlantic cod (*Gadus morhua*) and American plaice (*Hippoglossoides platessoides*). J. Northwest Atl. Fish. Sci. **11**: 39–42.

Hoag, S.H. 1975. Survival of halibut released after capture by trawls. Int. Pac. Halibut Comm. Sci. Rep. No. 57.

Horsted, S.A. 1963. On non-reported recaptures from Danish tagging experiments on cod, Subarea 1. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 4. pp. 22–25.

Horsted, S.A. 1965. Defects in the recovering and reporting of cod tagged by Denmark in Subarea 1. Int. Comm. Northwest Atl. Fish. Redb. 1965, Pt. III. pp. 172–177.

Horsted, S.A. 1967. Possible effect of a closure of Div. 1B to trawling judged by tagging experiments and other relevant data. Int. Comm. Northwest Atl. Fish. Res. Doc. No. 66/72.

McCracken, F.D. 1957. Results of cod tagging off eastern Nova Scotia. Fish. Res. Board Can. Prog. Rep. Atl. Coastal Stn. No. 67. pp. 6–13.

McCracken, F.D. 1959. Cod tagging off northern New Brunswick in 1955 and 1956. Fish. Res. Board Can. Prog. Rep. Atl. Coastal Stn. No. 72. pp. 8–19.

McCullagh, P., and Nelder, J.A. 1987. Generalized linear models. Chapman and Hall, London, U.K.

Myhre, R.J. 1966. Loss of tags from Pacific halibut, as determined by double-tag experiments. Int. Pac. Halibut Comm. Rep. No. 41.

Nielson, J. 1963. Comparison between different types of tags used on cod, char and salmon in Subarea 1. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 4. pp. 17–21.

Poulsen, E.M. 1957. Defects in the recovering of tagged fish. Int. Comm. Northwest Atl. Fish. Ser. No. 439(4).

Thorsteinsson, V. 1995. Tagging experiments using conventional tags and electronic data storage tags for the observations of migration, homing and habitat choice in the Icelandic spawning stock of cod. ICES CM 1995/B:19.

Trumble, R.J., McGregor, I.R., St-Pierre, G., McCaughran, D.A., and Hoag, S.H. 1990. Sixty years of tagging Pacific halibut: a case study. Am. Fish. Soc. Symp. **7**: 831–840.

Venables, W.N., and Ripley, B.D. 1994. Modern applied statistics with S-Plus. Springer-Verlag, New York.

Williams, T. 1963. Tests of efficiency of various kinds of tags and methods of attachment on plaice, cod, sole and whiting. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 4. pp. 156–163.

Wise, J.P. 1963. Factors affecting number and quality of returns from tagging cod with different tags and using different methods of capture in ICNAF divisions 4X and 5Y in 1957. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 4. pp. 101–105.

© 1999 NRC Canada

Appendix

Table A. Summary of releases and recoveries by species, modelling factor levels, and variate values.

			Cod			Haddock		
Effect	Type	Level	Releases	Recoveries	Recovery rate	Releases	Recoveries	Recovery rate
Total			81 499	16 352	0.201	43 999	2043	0.046
Immediate recoveries removed			78 431	13 283	0.169	43 694	1738	0.040
Minimum five or 10 releases per case			45 776	9 457	0.207	39 180	1352	0.035
Program	Factor	Historical	8 017	1 440	0.180	4 005	428	0.107
		Recent	39 857	5 919	0.149	35 175	924	0.026
Gear	Factor	Otter trawl	44 184	6 579	0.149	39 180	1352	0.035
		Longline	2 045	386	0.189	—	—	—
		Handline	690	203	0.294	—	—	—
		Cod trap	955	191	0.200	—	—	—
		Miscellaneous trap	—	—	—	—	—	—
		Angling	—	—	—	—	—	—
Tag	Factor	Dagger	—	—	—	—	—	—
		Lea hydrostatic	141	19	0.135	—	—	—
		Red–white Petersen	—	—	—	232	16	0.069
		Spaghetti	1 372	197	0.144	—	—	—
		T-bar	39 827	5 912	0.148	35 175	924	0.026
		French Petersen	397	148	0.373	—	—	—
		Petersen disc	4 492	800	0.178	3 374	409	0.121
		Red–yellow Petersen	—	—	—	—	—	—
		English–French Petersen	1 445	247	0.171	—	—	—
		Flagged Petersen	200	36	0.180	399	3	0.008
Area	Factor	3PN	—	—	—	—	—	—
		4SZ	867	153	0.176	—	—	—
		4TF	670	167	0.249	—	—	—
		4TG	8 104	1 111	0.137	374	41	0.110
		4TH	—	—	—	348	58	0.167
		4TK	568	92	0.162	—	—	—
		4TL	2 592	110	0.042	—	—	—
		4TM	84	43	0.512	—	—	—
		4TN	3 191	586	0.184	—	—	—
		4VC	5 749	1 200	0.209	—	—	—
		4VN	5 927	868	0.146	—	—	—
		4WD	—	—	—	—	—	—
		4WE	5 184	1 401	0.270	—	—	—
		4WF	1 882	38	0.020	—	—	—
		4WH	234	5	0.021	399	3	0.008
		4WJ	200	36	0.180	101	2	0.020
		4WK	162	15	0.093	—	—	—
		4WL	—	—	—	86	3	0.035
		4XM	184	28	0.152	—	—	—
		4XN	—	—	—	13 523	283	0.021
		4XO	399	51	0.128	209	47	0.225
		4XP	9 461	1 199	0.127	13 613	243	0.018
		4XR	—	—	—	3 612	439	0.122
		4XS	586	59	0.101	671	164	0.244
		5YB	—	—	—	195	0	0.000
		5ZJ	1 830	197	0.108	3 115	25	0.008
		5ZM	—	—	—	2 934	44	0.015
Month	Variate	Jan.	682	82	0.120	—	—	—

© 1999 NRC Canada

Pollock			Plaice		
Releases	Recoveries	Recovery rate	Releases	Recoveries	Recovery rate
56 873	5833	0.103	9512	1396	0.147
54 645	3605	0.066	9195	1079	0.117
53 159	3398	0.064	6565	622	0.095
—	—	—	1077	352	0.327
53 159	3398	0.064	5488	270	0.049
—	—	—	6565	622	0.095
—	—	—	—	—	—
—	—	—	—	—	—
24 052	2552	0.106	—	—	—
23 802	623	0.026	—	—	—
5 305	223	0.042	—	—	—
—	—	—	202	38	0.188
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
52 237	3273	0.063	1593	87	0.055
—	—	—	—	—	—
922	125	0.136	3980	189	0.047
—	—	—	790	308	0.390
—	—	—	—	—	—
—	—	—	—	—	—
306	29	0.095	—	—	—
—	—	—	—	—	—
—	—	—	173	45	0.260
—	—	—	3024	245	0.081
—	—	—	—	—	—
—	—	—	194	16	0.082
—	—	—	2067	182	0.088
—	—	—	68	46	0.676
—	—	—	220	61	0.277
—	—	—	—	—	—
—	—	—	—	—	—
20 871	2036	0.098	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
4 849	102	0.021	89	0	0.000
—	—	—	—	—	—
—	—	—	645	21	0.033
—	—	—	—	—	—
5 651	520	0.092	52	4	0.077
—	—	—	—	—	—
5 831	222	0.038	—	—	—
15 651	489	0.031	33	2	0.061
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
291	0	0.000	—	—	—

© 1999 NRC Canada

Table A (*continued*).

Effect	Type	Level	Cod			Haddock		
			Releases	Recoveries	Recovery rate	Releases	Recoveries	Recovery rate
		Feb.	6 970	943	0.135	2 136	12	0.006
		Mar.	5 188	589	0.114	25 070	382	0.015
		Apr.	7 083	1 222	0.173	6 420	205	0.032
		May	3 589	708	0.197	—	—	—
		June	1 427	161	0.113	3 043	368	0.121
		July	681	153	0.225	305	1	0.003
		Aug.	468	69	0.147	—	—	—
		Sept.	8 835	802	0.091	477	59	0.124
		Oct.	5 515	979	0.178	547	88	0.161
		Nov.	5 414	1 280	0.236	973	190	0.195
		Dec.	2 022	371	0.183	209	47	0.225
Year	Variate	1955	549	170	0.310	—	—	—
		1956	—	—	—	722	99	0.137
		1957	114	27	0.237	1 099	198	0.180
		1958	1 963	346	0.176	—	—	—
		1959	432	53	0.123	187	5	0.027
		1960	1 204	168	0.140	399	3	0.008
		1961	1 604	305	0.190	—	—	—
		1962	353	92	0.261	—	—	—
		1963	—	—	—	507	63	0.124
		1964	925	165	0.178	—	—	—
		1966	92	23	0.250	285	47	0.165
		1969	682	82	0.120	145	4	0.028
		1971	—	—	—	661	9	0.014
		1972	—	—	—	—	—	—
		1973	99	9	0.091	—	—	—
		1978	5 184	1 401	0.270	—	—	—
		1979	5 181	520	0.100	—	—	—
		1980	8 775	1 430	0.163	—	—	—
		1981	9 031	1 145	0.127	—	—	—
		1982	—	—	—	2 898	364	0.126
		1983	—	—	—	3 370	183	0.054
		1984	5 458	745	0.136	13 842	263	0.019
		1985	6 228	678	0.109	15 065	114	0.008
Group size	Variate	5 (10) – 400	8 056	1 302	0.162	5 482	459	0.084
		401–800	11 120	1 906	0.171	372	6	0.016
		801–1200	12 966	2 193	0.169	—	—	—
		1201–1600	10 566	1 289	0.122	—	—	—
		1601–2000	3 119	372	0.119	1 937	12	0.006
		2001–2400	2 047	297	0.145	2 202	39	0.018
		2401–2800	—	—	—	10 521	206	0.020
		2801–3200	—	—	—	11 601	441	0.038
		3201–3600	—	—	—	3 195	54	0.017
		3601–4000	—	—	—	3 870	135	0.035
Depth	Variate	0–19	556	140	0.252	—	—	—
		20–37	3 771	967	0.256	3 458	446	0.129
		38–55	15 852	2 317	0.146	482	115	0.239
		56–74	7 199	1 158	0.161	748	21	0.028
		75–92	14 837	1 849	0.125	18 996	496	0.026
		93–110	4 317	682	0.158	15 426	269	0.017
		111–128	685	119	0.174	70	5	0.071
		129–147	646	124	0.192	—	—	—
		148–165	11	3	0.273	—	—	—

© 1999 NRC Canada

Pollock			Plaice		
Releases	Recoveries	Recovery rate	Releases	Recoveries	Recovery rate
193	0	0.000	734	21	0.029
98	0	0.000	—	—	—
—	—	—	52	4	0.077
19 680	786	0.040	676	97	0.143
15 659	1748	0.112	1204	247	0.205
3 359	171	0.051	—	—	—
1 665	93	0.056	141	38	0.270
1 490	16	0.011	3500	170	0.049
4 151	489	0.118	253	45	0.178
4 517	87	0.019	5	0	0.000
2 056	8	0.004	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	414	164	0.396
—	—	—	317	124	0.391
—	—	—	194	35	0.180
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	67	23	0.343
—	—	—	—	—	—
—	—	—	33	2	0.061
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	52	4	0.077
—	—	—	—	—	—
922	125	0.136	—	—	—
11 118	1499	0.135	3500	170	0.049
11 986	170	0.014	1988	100	0.050
6 882	677	0.098	—	—	—
8 274	456	0.055	—	—	—
9 990	218	0.022	—	—	—
3 987	253	0.063	—	—	—
—	—	—	—	—	—
2 283	55	0.024	4846	586	0.121
4 572	96	0.021	1719	36	0.021
2 804	127	0.045	—	—	—
2 922	40	0.014	—	—	—
5 522	37	0.007	—	—	—
6 640	264	0.040	—	—	—
5 230	658	0.126	—	—	—
8 356	954	0.114	—	—	—
—	—	—	—	—	—
14 830	1167	0.079	—	—	—
53 159	3398	0.064	—	—	—
—	—	—	170	72	0.424
—	—	—	1939	281	0.145
—	—	—	3016	121	0.040
—	—	—	1119	81	0.072
—	—	—	298	67	0.225
—	—	—	23	0	0.000
—	—	—	—	—	—
—	—	—	—	—	—

© 1999 NRC Canada

Table A (*concluded*).

Effect	Type	Level	Cod			Haddock		
			Releases	Recoveries	Recovery rate	Releases	Recoveries	Recovery rate
Length	Variate	11–20	—	—	—	6	0	0.000
		21–30	118	10	0.085	1 204	84	0.070
		31–40	6 064	543	0.090	3 253	319	0.098
		41–50	17 838	2 930	0.164	11 845	441	0.037
		51–60	12 667	2 237	0.177	15 757	414	0.026
		61–70	5 308	798	0.150	6 244	87	0.014
		71–80	2 965	417	0.141	855	7	0.008
		81–90	1 878	286	0.152	16	0	0.000
		91–100	841	117	0.139	—	—	—
		101–110	185	20	0.108	—	—	—
		111–120	10	1	0.100	—	—	—

Note: The variates are grouped for presentation but not for input to the modelling exercise.

© 1999 NRC Canada

Pollock			Plaice		
Releases	Recoveries	Recovery rate	Releases	Recoveries	Recovery rate
23 752	513	0.022	149	1	0.007
19 886	1207	0.061	1722	40	0.023
8 184	1351	0.165	3205	306	0.095
1 337	327	0.245	1332	239	0.179
—	—	—	105	24	0.229
—	—	—	52	12	0.231
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—

© 1999 NRC Canada

Lake sturgeon (*Acipenser fulvescens*) growth chronologies

Gregory T.O. LeBreton, F. William H. Beamish, and Robert G. Wallace

Abstract: We used annual increments in the pectoral fin rays of lake sturgeon (*Acipenser fulvescens*) to assess growth synchrony. We based our decision criteria on correlation among individual chronologies developed from samples collected in the Saskatchewan River, Saskatchewan, and Lake St. Clair and the Mattagami River, Ontario. Initially, correlations among chronologies were not significant within these three populations. However, as mean ageing error was reduced, correlations among chronologies increased to significant levels in samples from the Saskatchewan River and Lake St. Clair. Correlations were not significant among consistently aged fish sampled from the Mattagami River. We conclude that interannual growth variation in lake sturgeon is influenced by population-wide extrinsic factors in some populations. Our results suggest that both growth synchrony and ageing error should be quantified during the construction of growth chronologies for all organisms.

Résumé : Nous avons utilisé les incréments annuels des rayons des nageoires pectorales de l'esturgeon jaune (*Acipenser fulvescens*) pour évaluer la synchronie de la croissance. Nous avons fondé nos critères de décision sur la corrélation entre les chronologies individuelles établies dans des échantillons recueillis dans la rivière Saskatchewan (Saskatchewan) et dans le lac Sainte-Claire et la rivière Mattagami (Ontario). Initialement, les corrélations entre les chronologies n'étaient pas significatives à l'intérieur de ces trois populations. Cependant, avec la réduction de l'erreur moyenne d'âgeage, les corrélations entre les chronologies se sont accrues pour devenir significatives dans les échantillons de la rivière Saskatchewan et du lac Sainte-Claire. Les corrélations n'étaient pas significatives chez les poissons de la Mattagami dont on a systématiquement déterminé l'âge. Nous concluons que, dans certaines populations, la variation interannuelle de la croissance chez les esturgeons jaunes est tributaire de facteurs extrinsèques touchant l'ensemble de la population. Nos résultats indiquent qu'on devrait quantifier autant la synchronie de la croissance que l'erreur d'âgeage quand on établit des chronologies de la croissance, et ce, pour tous les organismes.

[Traduit par la Rédaction]

Introduction

Growth of exothermic organisms is influenced by extrinsic factors. If these factors operate over large geographic scales, their influence will be exerted on all members of a population (Thompson and Page 1989). Growth of exothermic organisms is also influenced by intrinsic physiological factors. These factors, such as health and reproductive cycles, do not similarly influence all members of a population but operate independently on individuals. A population of exotherms, most influenced by large-scale extrinsic factors, will display synchronous interannual variations in growth among its members (Thompson and Page 1989). However, if growth is most influenced by intrinsic factors, interannual growth variation may be asynchronous throughout this population.

Growth chronologies are time series that display annual growth fluctuations in individuals or populations over a series of calendar years. Constructed from rings or annuli in the hard tissues of exotherms, growth chronologies usually have the effects of age on growth mathematically removed (Fritts 1976; Ogle et al. 1994; Pereira et al. 1995; Cyterski and Spangler 1996). These chronologies provide insight into the ecology of a species (Pereira et al. 1995), allow for the development of predictive models (Cyterski and Spangler 1996), and may assist in the determination of factors influencing growth. However, to develop a growth chronology for a population, all members of that population must be responding to a similar set of growth-influencing factors. For example, if growth is most influenced by population-wide extrinsic factors, individual growth chronologies will display synchronous variation and be highly correlated among members of that population. Conversely, individual growth chronologies most influenced by intrinsic factors may display asynchrony, show no correlation among population members, and therefore display no significant growth variations when averaged into population chronologies.

The lake sturgeon (*Acipenser fulvescens*) is found in the estuarial waters of the St. Lawrence River as far west as the North and South Saskatchewan rivers in Alberta and north to the Nelson River in Manitoba. Individuals may exceed 100 years of age (Houston 1987). Growth rings in the pectoral fin rays of this species are annually developed (Rossiter et al. 1995). These characteristics make this species an exceptional candidate with which to investigate the synchrony of interannual growth variations in different populations. In-

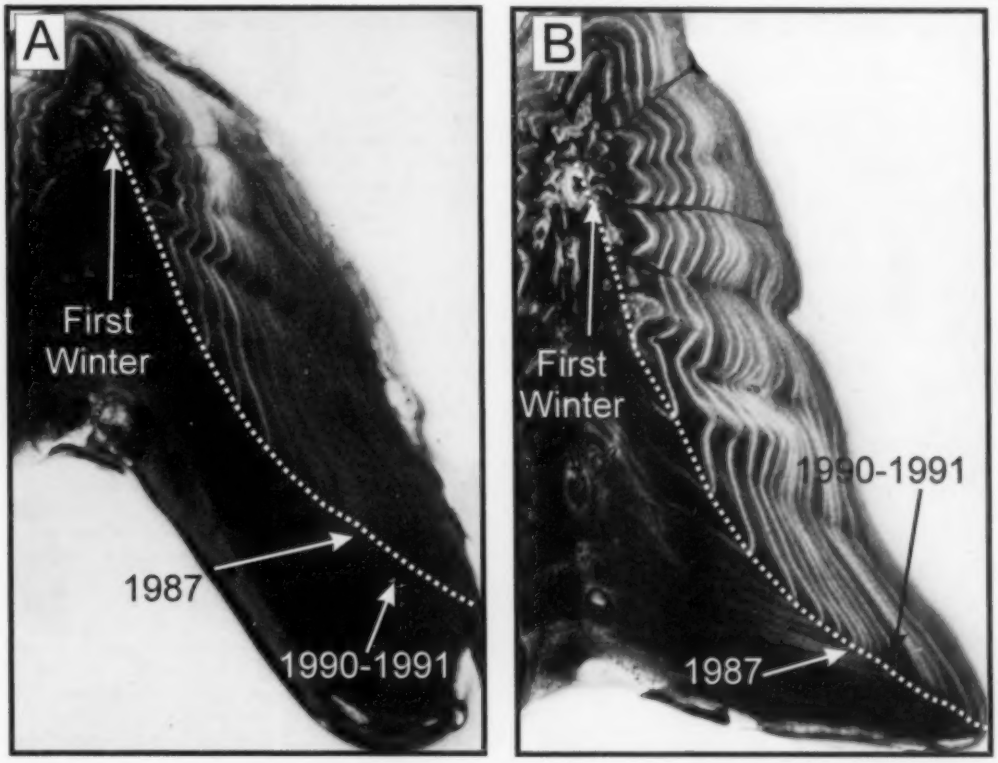
Received September 1, 1998. Accepted March 26, 1999. J14763

G.T.O. LeBreton[1] **and F.W.H. Beamish.** Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada.
R.G. Wallace. Saskatchewan Environment and Resource Management, 112 Research Drive, Saskatoon, SK S7K 2H6, Canada.

[1]Author to whom all correspondence should be addressed. e-mail: glebreto@uoguelph.ca

© 1999 NRC Canada

Fig 1. Cross sections of lake sturgeon pectoral fin rays. (A) 1973 year-class, age = 23; (B) 1977 year-class, age = 19. Years indicated by arrows are periods of synchronous growth variation. Dotted lines indicate the radius over which increment widths were measured.



terestingly, there is debate regarding whether extrinsic or intrinsic factors most influence growth in this species. Some research indicates that lake sturgeon growth is controlled by intrinsic factors such as gonadogenesis (Roussow 1957). However, this concept has not been substantiated by recent investigations (Guénette et al. 1992). In the current investigation, we developed growth chronologies from annual increments in pectoral fin rays of lake sturgeon. We used the interseries correlation coefficient (Wigley et al. 1984) and Monte Carlo simulations (Prager and Hoenig 1989; Edgington 1995) to determine if interannual growth variations display significant synchrony within three populations.

Materials and methods

We obtained lake sturgeon pectoral fin rays from archived collections of fish sampled from the lower Saskatchewan River, Saskatchewan (53°54′N, 102°20′W), between 1978 and 1982 (Wallace 1991), from Lake St. Clair, Ontario (42°00′N, 82°30′W), between 1991 and 1996, and from the Mattagami River, Ontario (49°55′N, 81°37′W), during 1996 and 1997. Rays had been previously prepared, sectioned to about 250 μm, and mounted on glass microscope slides.

We used the widths of growth increments, the consecutive pairs of opaque and translucent zones in each fin ray's cross section, as a record of past growth for each individual (Wilson 1987; Rossiter et al. 1995). Sections that were too opaque or translucent to read were excluded from the analysis. Similarly, we excluded sections that displayed signs of breakage. This condition, described in detail by Wilson (1987), is caused when fins become broken during upstream spawning migrations and is identified by regions of discontinuous rings in the cross section. We measured widths of growth increments using a compound microscope (40×) with a drawing tube situated over a digitizing tablet. Increment measurements were made along the most legible posterior radius (Fig. 1). In total, 48, 58, and 108 samples were analyzed from the Saskatchewan River, Lake St. Clair, and Mattagami River, respectively.

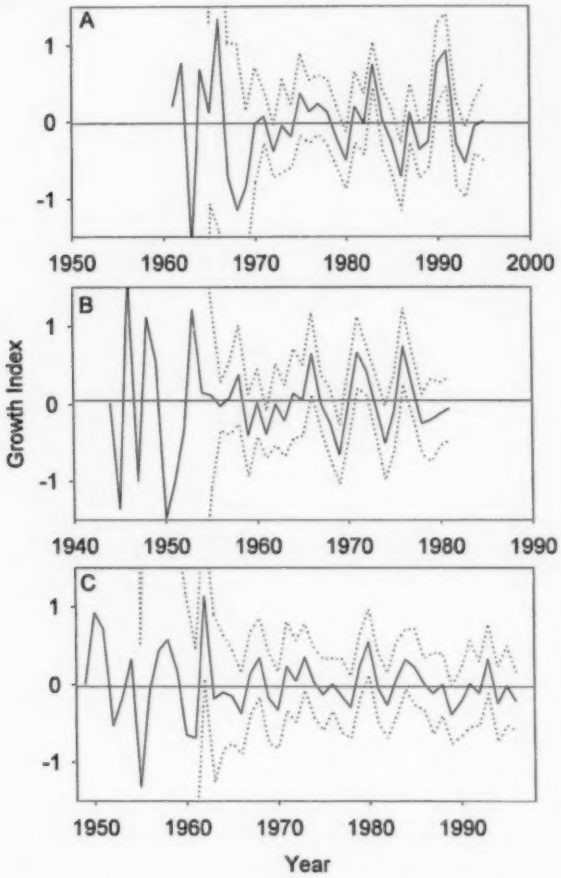
For each fin ray sample, increment widths were measured in three blind replicates. From these replicates, we calculated the index of ageing error, a measure of the inability to consistently age a sample (Beamish and Fournier 1981). For all samples the last complete growth increment was assumed to have developed during the calendar year prior to capture, and all increments were dated with respect to this year.

To construct chronologies, we adjusted increment widths for the decrease in relative growth with age using a technique common to the analysis of tree rings by dendrochronologists (Fritts 1976). We define an individual chronology as a series of growth data collected from an individual fish for which the decrease in relative growth with age has been removed. A population chronology is a series of growth data constructed by averaging numerous individual chronologies, aligned by calendar year, from a population.

We constructed an individual chronology by calculating a 7-year running average from the series of growth increment widths measured from a fin ray sample. This curve approximated the decrease in increment width with age. We then calculated the residuals, the difference between an increment width and its corresponding running average value. Each residual approximated annual growth relative to that in the 3 years preceding and following it. The variance

© 1999 NRC Canada

Fig 2. Lake sturgeon population chronologies of consistenly aged fish from (A) Lake St. Clair, (B) Saskatchewan River, and (C) Mattagami River. Dotted lines indicate approximate 95% confidence intervals. Solid horizontal lines indicate chronology means.



of these residuals also decreased with age (Maceina 1992). We homogenized the variance through each individual chronology as follows. Residuals were converted to absolute values and each series was fit with another 7-year running average. This curve approximated the decrease in variance of the residuals with age. Each residual, with its original sign (+ or –), was then divided by its corresponding running average value. Remaining age-related trends were detected by aligning and averaging chronologies from each population by age. We removed these age-related trends by subtracting the average curves from each individual chronology. The resulting individual chronologies displayed annual growth fluctuations, measured in unitless growth indices, with a homogeneous variance fluctuating about a mean of zero. We constructed population chronologies by averaging individual chronologies. From this, average calendar year indices of growth and approximate 95% confidence intervals were calculated.

Ageing error, the inability to consistently age a calcified tissue sample, misaligns and disrupts patterns among assembled individual chronologies, thereby influencing the synchrony of growth variation. This effect of ageing error was determined by analyzing correlations among all individual chronologies and then assemblages of chronologies with mean indices of ageing error equal to 0.005 and 0.000.

Correlations among chronologies were assessed using the interseries correlation coefficient (Wigley et al. 1984). This statistic assesses the mean correlation among all possible pairs of chronologies and ranges from about zero, if growth fluctuations are completely asynchronous, to 1, if growth fluctuations are completely synchronous. The interseries correlation coefficient was calculated for a set of individual chronologies using a two-way analysis of variance applied to growth data organized with chronologies in rows and calendar year in columns. Correlations were investigated for data for 1965–1978, 1977–1990, and 1982–1995 from samples from the Saskatchewan River, Lake St. Clair, and Mattagami River populations, respectively. Samples not completely spanning these periods were excluded from the analysis.

We calculated the null distribution of the interseries correlation coefficient using Monte Carlo simulations. To estimate the distribution of coefficients, we repeatedly generated random sets of chronologies that were similar in their mean, variance, length, and number to those extracted from lake sturgeon samples. By generating 1000 sets of these random chronologies and calculating the interseries correlation coefficient for each set, we created a null distribution for this statistic.

The level of significance (p value) for each correlation coefficient that was calculated from sets of lake sturgeon growth chronologies was determined by setting x equal to the number of sets of chronologies generated in the Monte Carlo simulation that have coefficients greater than the calculated test statistic and setting y equal to the total number of sets generated. The resulting significance level is calculated from $(x + 1)/(y + 1)$. A value of 1 is added to both the numerator and denominator of this equation, as the calculated test statistic is included in the estimated null distribution (Edgington 1995). For example, the interseries correlation coefficient of the 23 lake sturgeon chronologies from the Saskatchewan River that displayed no ageing error and spanned 1965–1978 was 0.1133. To calculate the null distribution for this statistic, we generated 1000 sets of 23 random chronologies, 14 years in length, and calculated interseries correlation coefficients for each set. Three of these artificially generated coefficients were greater than 0.1133. Therefore, the level of significance (p) was calculated as $(3 + 1)/(1000 + 1) = 0.0040$. We determined the critical value (p) of our nine calculated test statistics such that $\alpha = 0.05$ to be $\leq 5.56 \times 10^{-3}$ based on the Bonferroni method of multiple comparisons (α/k) (Judd and McClelland 1989).

Results

The correlation coefficient among all 58 chronologies from Lake St. Clair was 0.0407 ($p = 0.049$). The mean age and index of ageing error of these samples were 25.2 years and 0.021, respectively. When the mean index of ageing error was reduced to 0.005 and then to 0.000, the corresponding correlation coefficients increased to 0.0926 ($n = 37$, mean age = 24.2, $p = 0.003$) and 0.1445 ($n = 22$, mean age = 22.1, $p < 0.001$), respectively. The population chronology assembled using 22 consistently aged samples spanned 1961–1995 and displayed significant growth variations during 1974, 1983, 1985–1988, 1990–1991, and 1994 (Fig. 2A). The confidence intervals throughout the early years of all chronologies are wide, as fewer data points were available from older fish.

All 48 lake sturgeon chronologies from the Saskatchewan River displayed a correlation coefficient of 0.0581 ($p = 0.023$) and a mean age of 25.0 years. The mean index of ageing error among these samples was 0.012. When the mean index of ageing error was reduced to 0.005 and then to 0.000, the corresponding correlation coefficients increased

© 1999 NRC Canada

to 0.1044 (n = 34, mean age = 26.0, p = 0.003) and 0.1133 (n = 23, mean age = 25.1, p = 0.004), respectively. The population growth chronology assembled using the 23 consistently aged samples spanned 1944–1981. Growth indices in this chronology deviated significantly from the mean during 1961, 1965, 1969, 1971, 1972, 1974, and 1976 (Fig. 2B).

The correlation coefficient among 108 chronologies from the Mattagami River was 0.0075 (p = 0.261). The mean age and index of aging error of these samples were 31.6 years and 0.0242, respectively. When then mean index of ageing error was reduced to 0.005 and then to 0.000, the corresponding correlation coefficients were 0.0075 (n = 53, mean age = 31.4, p = 0.340) and 0.0037 (n = 32, mean age = 28.1, p = 0.694) respectively. The population chronology developed from the 32 consistently aged samples spanned 1952–1996. Growth indices in this chronology deviated significantly from the mean during 1973, 1980, and 1989 (Fig. 2C).

Discussion

Sturgeon chronologies, developed from consistently aged individuals, indicated significantly synchronous growth variations among fish from the Saskatchewan River and Lake St. Clair populations. No significant correlations were detected among chronologies from Mattagami River fish. We found ageing error to disrupt growth patterns and significantly reduce correlation among chronologies in all populations. By removing inconsistently aged individuals from our analysis, we enhanced the strength of the common signal contained in each population chronology (Wigley et al. 1984). As correlation among individual chronologies increased, numbers of years during which growth differed significantly from the mean also increased.

From these results, we conclude that lake sturgeon growth is influenced by population-wide extrinsic factors in the Saskatchewan River and Lake St. Clair. We found no such evidence for fish from the Mattagami River. One extrinsic factor responsible for controlling lake sturgeon growth throughout a population may be temperature. Air temperature was found to suitably predict average growth rates in a comparison of 32 lake sturgeon populations (Fortin et al. 1996). Roussow (1957), investigating growth patterns in lake sturgeon annuli, indicated that growth was controlled by intrinsic physiological factors such as health and spawning periodicity. Our results indicate that such factors did not significantly influence lake sturgeon growth in Lake St. Clair or the Saskatchewan River. In our study, lake sturgeon averaging 25 years of age displayed significantly synchronous growth fluctuations in these populations. Our results for these populations do support the findings of Guénette et al. (1992), who found a lack of correlation between state of maturity and the widths of the last five growth increments in lake sturgeon from the St. Lawrence River. Their research also suggested that extrinsic factors may influence interannual growth variation.

Relative to other exothermic organisms, lake sturgeon display relatively low synchrony of interannual growth variations. Wigley et al. (1984) reported the interseries correlation coefficients for several studies conducted on tree rings. Correlations for these terrestrial populations of exotherms ranged from 0.1779 (n = 13, years = 100) to 0.5297 (n = 18, years = 100). No levels of significance were reported with these statistics.

Recently, growth chronologies have been constructed for several varied groups of organisms from molluscs and mammals to fish and trees (Jones 1980; Boyd and Roberts 1993; Guyette and Rabeni 1995). These time series improve our understanding of environmental factors influencing growth and assist prediction of population productivity (Fritts 1976; Ogle et al. 1994; Guyette and Rabeni 1995; Pereira et al. 1995; Cyterski and Spangler 1996). From the current investigation, we conclude that the development of growth chronologies for any organism must be approached with caution for several reasons. Chronologies constructed from populations that fail to display synchronous interannual growth variations, such as Mattagami River lake sturgeon, may result in poorly developed chronologies and incorrect predictive models. Furthermore, ageing errors must be minimized to ensure that patterns in population growth chronologies are correct and that the common signals contained in these are maximized.

Acknowledgements

We thank D. MacLennan and R.S. McKinley for supplying archived samples from Lake St. Clair and the Mattagami River, respectively, and Dr. D.L.G. Noakes for his critical review of this manuscript. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada to F.W.H.B.

References

Beamish, R.J., and Fournier, D.A. 1981. A method for the comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. **38**: 982–983.

Boyd, I.L., and Roberts, J.P. 1993. Tooth growth in male Antarctic fur seals (*Arctocephalus gazella*) from South Georgia: an indicator of long-term growth history. J. Zool. (Lond.), **229**: 177–190.

Cyterski, M.J., and Spangler, G.R. 1996. Development and utilization of a population growth history of Red Lake walleye, *Stizostedion vitreum*. Environ. Biol. Fishes, **46**: 45–49.

Edgington, E.S. 1995. Randomization tests. Marcel Dekker, Inc., New York.

Fortin, R., Dumont, P., and Guénette, S. 1996. Determinants of growth and body condition of lake sturgeon (*Acipenser fulvescens*). Can. J. Fish. Aquat. Sci. **53**: 1150–1156.

Fritts, H.C. 1976. Trees rings and climate. Academic Press, New York.

Guénette, S., Goyette, D., Fortin, R., Leclerc, J., Fournier, N., Roy, G., and Dumont, P. 1992. La périodicité de la croissance chez la femelle de l'esturgeon jaune (*Acipenser fulvescens*) du fleuve Saint-Laurent est-elle reliée à la périodicité de la reproduction? Can. J. Fish. Aquat. Sci. **49**: 1336–1342.

Guyette, R.P., and Rabeni, C.F. 1995. Climate response among growth increments of fish and trees. Oecologia, **104**: 272–279.

Houston, J.J. 1987. Status of the lake sturgeon, *Acipenser fulvescens*, in Canada. Can. Field-Nat. **101**: 171–185.

Jones, S.J. 1980. Annual growth increments in shells of *Spisula solidissima* record marine temperature variability. Science (Washington, D.C.), **211**:165–166.

Judd, C.M., and McClelland, G.H. 1989. Data analysis. A model comparison approach. Harcourt Brace Jovanovich, New York.

© 1999 NRC Canada

Maceina, M.J. 1992. A simple regression model to assess environmental effects on fish growth. J. Fish Biol. **41**: 557–565.

Ogle, D.H., Spangler, G.R., and Shroyer, S.M. 1994. Determining fish age from temporal signatures in growth increments. Can. J. Fish. Aquat. Sci. **51**: 1721–1727.

Pereira, D.L., Bingham, C., Spangler, G.R., Cohen, Y., Connor, D.J., and Cunningham, P.K. 1995. Growth and recruitment of freshwater drun (*Aplodinotus grunniens*) as related to long term temperature patterns. *In* Climate change and northern fish populations. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 617–629.

Prager, M.H., and Hoenig, J.M. 1989. Superposed epoch analysis: a randomization test of environmental effects on recruitment with applications to chub mackerel. Trans. Am. Fish. Soc. **118**: 608–618.

Rossiter, A., Noakes, D.L.G., and Beamish, F.W.H. 1995. Validation of age estimation for the lake sturgeon. Trans. Am. Fish. Soc. **124**: 777–781.

Roussow, G. 1957. Some considerations concerning sturgeon spawning periodicity. J. Fish. Res. Board Can. **14**: 553–572.

Thompson, K.R., and Page, F.H. 1989. Detecting synchrony of recruitment using short autocorrelated time series. Can. J. Fish. Aquat. Sci. **46**: 1831–1838.

Wallace, R.G. 1991. Species recovery plan for lake sturgeon in the lower Saskatchewan River (Cumberland Lake area). Fish. Tech. Rep. No 91-3. Saskatchewan Parks and Renewable Resources, Fisheries Branch, Saskatoon, Sask.

Wigley, T.M.L., Briffa, K.R., and Jones, P.D. 1984. On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. J. Clim. Appl. Meteorol. **23**: 201–213.

Wilson, N.C. 1987. Age determination of lake sturgeon (*Acipenser fulvescens*) by use of the marginal pectoral fin ray. *In* Proceedings of a Workshop on the Lake Sturgeon (*Acipenser fulvescens*). *Edited by* C.H. Oliver. Ont. Fish. Tech. Rep. Ser. No. 23. pp. 77–83.

© 1999 NRC Canada

Effets des métaux (Ag, Cd, Cu) sur la composition biochimique et compartimentation de ces métaux chez deux microalgues *Skeletonema costatum* et *Tetraselmis suecica*

H. Perrein-Ettajani, J.C. Amiard, J. Haure et C. Renaud

Résumé : Les effets des concentrations subléthales d'argent, de cadmium et de cuivre sur la croissance et la composition biochimique de deux microalgues marines *Tetraselmis suecica* et *Skeletonema costatum* révèlent que la croissance est de loin le facteur le plus sensible, tandis que les concentrations cellulaires en polysaccharides, en protéines, en lipides et en pigments totaux ne sont pas significativement affectées. Ceci suggère que la première cible de ces métaux est la division cellulaire et les processus qui lui sont liés. Les concentrations métalliques dans les cellules algales sont plus faibles chez *S. costatum* que chez *T. suecica*, et l'étude de la répartition des métaux montre que pour les deux espèces d'algues, le cadmium est fortement lié aux parois cellulaires ou a pénétré dans la cellule, tandis que 40% du cuivre initialement adsorbé est aisément désorbable après traitement avec l'agent chélatant. Toutefois, chez les deux espèces algales, le cadmium et le cuivre sont présents de façon majoritaire dans la fraction insoluble, tandis que dans le cytosol, ce sont des polypeptides thermorésistants qui sont sollicités. Dans cette étude, la voie principale de détoxication chez les algues exposées à des concentrations métalliques subléthales est donc leur liaison avec des composés insolubles. La forte stabilité de ces derniers suggère une faible biodisponibilité et vraisemblablement un transfert limité de ces métaux vers les niveaux trophiques supérieurs.

Abstract: Effects at sublethal silver, cadmium, and copper concentrations on growth and biochemical composition of two marine microalgae *Tetraselmis suecica* and *Skeletonema costatum* revealed that growth is by far the most sensitive factor, whereas cellular content of polysaccharides, proteins, lipids, and pigments are not significantly affected. This suggests that the first target of these metals is cell division and processes related to it. Heavy metal concentrations in algal cells were lower in *S. costatum* than in *T. suecica*, and the study of metal partitioning showed that for both algal species, Cd was firmly bound onto the cell wall or had entered the cell, whereas 40% of the initially adsorbed Cu was easily released after treatment by the chelating agent. However, in both algal species, Cd and Cu were predominantly present in the insoluble fraction, and in the cytosol, thermoresistant metal-chelating polypeptids are involved. For this study, the major metal detoxification process in algae exposed to sublethal concentrations is therefore their binding to insoluble components. The strong stability of the later suggests less bioavailability and maybe a limited transfer of these metals to higher trophic levels.

Introduction

Dans les milieux aquatiques, le phytoplancton constitue la base des réseaux trophiques et, d'autre part, présente souvent une grande capacité d'accumulation à l'égard de nombreux polluants dispersés dans l'environnement. Cette phase particulaire est un vecteur non négligeable de transfert des éléments métalliques dans les chaînes trophiques (Amiard et al. 1989; Ettajani et al. 1992).

Continuellement exposées en milieu estuarien aux polluants métalliques tels que l'Ag, le Cd et le Cu, les microalgues ont développé différents mécanismes de tolérance. En effet, les métaux peuvent s'adsorber à la surface algale ou à la matière organique excrétée par les algues (Brown et al. 1988; Visviki et Rachlin 1994), ou encore être liés au niveau du cytosol, à des composés protéiniques tels que les phytochélatines (Ahner et al. 1994) ou dans des organites intracellulaire (Ballan-Dufrançais et al. 1991). La détoxication permet d'éviter ou du moins de réduire la toxicité de ces éléments sur la croissance et les processus physiologiques tels que la photosynthèse, la respiration, l'absorption d'éléments nutritifs. Les effets des métaux sur ces paramètres ont été largement étudiés (Stauber et Florence 1990). Toutefois, les surcharges métalliques choisies sont souvent très élevées par rapport à celles rencontrées dans des milieux contaminés, alors que la réponse des algues à certains polluants peut être

Reçu le 24 septembre 1998. Accepté le 16 avril 1999.
J14795

H. Perrein-Ettajani. Institut d'écologie appliquée, UCO, 3 place André Leroy, B. P. 808, 49008 Angers Cédex 1, et Centre national de la recherche scientifique (CNRS), Groupement de recherche, 1117 et Université de Nantes, Service d'écotoxicologie, ISOMer, 1, rue Gaston Veil, F-44035 Nantes Cédex, France.
J.C. Amiard et C. Renaud. Centre national de la recherche scientifique (CNRS), Groupement de recherche, 1117 et Université de Nantes, Service d'écotoxicologie, ISOMer, 1, rue Gaston Veil, F-44035 Nantes Cédex, France.
J. Haure. Institut français de recherche pour l'exploitation de la mer, Polders des Champs, 85230 Bouin, France.

© 1999 NRC Canada

adverse selon que la concentration est subléthale ou aiguë. Visviki et Rachlin (1992) l'ont souligné pour *Dunaliella minuta* : la quantité de lipides diminue quand les concentrations en Cd sont aiguës, elle augmente toutefois quand celles-ci sont subléthales.

Le rôle inhibiteur de certains métaux sur la croissance algale a été largement étudié (Metaxas et Lewis 1991; Visviki et Rachlin 1994). De plus, compte tenu de sa grande sensibilité vis-à-vis de ces éléments, la croissance constitue le paramètre le plus classiquement utilisé pour les essais de toxicité. Nyholm et Damgaard (1990) ont montré que pour *Selenastrum capricornutum*, les bioessais basés sur la croissance rendent compte des effets chroniques des métaux, alors que ceux basés sur la photosynthèse sont considérés mesurer la toxicité aiguë.

Le but de cette étude est de caractériser d'une part, les perturbations aussi bien au niveau de la croissance qu'au niveau de la composition métabolique des deux microalgues *Skeletonema costatum* et *Tetraselmis suecica* soumises à des concentrations subléthales d'Ag, de Cd ou de Cu, et de déterminer d'autre part, les mécanismes de détoxication sollicités par les deux algues. Le choix de l'Ag, du Cd et du Cu pour cette étude réside dans le fait que ces éléments se trouvent à des concentrations élevées suite aux rejets anthropogéniques. Des réseaux de surveillance (RNO 1995) montrent qu'ils sont bioaccumulés par les organismes aquatiques. De plus, pour l'Ag, peu d'études ont abordé la toxicité de cet élément. Ces deux espèces (*Skeletonema costatum* et *Tetraselmis suecica*) ont été choisies, car elles sont largement employées en conchyliculture du fait de leurs bonnes valeurs nutritionnelles et leur facilité de culture. De plus, *S. costatum* est présente presque toute l'année et en abondance dans les claires ostréicoles de la baie de Bourgneuf et constitue un maillon important des chaînes trophiques à mollusques de cette baie.

Une méthodologie a été développée (Amiard-Triquet et al. 1992) permettant d'étudier la répartition des métaux entre les formes solubles (dont les phytochélatines et les enzymes) et insolubles (concrétions minérales, groupements membranaires). Cette méthodologie, outre les prévisions qu'elle autorise en terme de spéciation et de biodisponibilité des métaux au maillon suivant (Ettajani et Pirastru 1992), permet également de prévoir les effets biologiques de ces derniers : parmi les composés liant les métaux, les composés thermorésistants incluent principalement les phytochélatines, tandis que les enzymes qui sont les cibles moléculaires des métaux sont présents dans la phase thermosensible.

Matériels et méthodes

Culture et contamination des microalgues

Les souches de *Skeletonema costatum* et de *Tetraselmis suecica* ont été fournies respectivement par la station Institut français de recherche pour l'exploitation de la mer de Bouin (France) et par l'algothèque du laboratoire de Biologie Marine (Nantes, France).

Avant la mise en culture des algues, l'espèce *T. suecica* a nécessité une acclimatation progressive à l'eau souterraine. Cette dernière, naturellement riche en nutriments, est largement utilisée en baie de Bourgneuf (Vendée, France) pour les cultures phytoplanctoniques. Pour *S. costatum* (souche Bouin 1), l'inoculum constitué de cellules en phase de division active est prélevé d'un bac de culture (50 m^3) servant de nourriture en nourricerie des mollusques.

Les deux microalgues ont été cultivées en grand volume (600 L) dans de l'eau souterraine salée au mois de mai sous des conditions naturelles d'éclairement et de température. De plus, un bullage continu d'air a été utilisé pour homogénéiser les cultures et éviter ainsi la sédimentation des cellules. Ces cultures en grands volumes ont été réalisées dans le but de disposer de biomasse algale suffisante pour des expériences ultérieures de transfert des métaux aux huîtres.

Les différents contaminants Ag, Cd et Cu sont introduits dans les différents milieux de cultures sous forme de solution nitrique 0,5 $mol \cdot L^{-1}$, le 1er jour d'ensemencement et renouvelés le 5^{e} jour de culture. Les concentrations des solutions mères des métaux sont choisies afin d'éviter toute modification du pH du milieu.

Les cultures d'algues ont été contaminées par 20 $\mu g\ Ag \cdot L^{-1}$, 20 $\mu g\ Cd \cdot L^{-1}$ et 50 $\mu g\ Cu \cdot L^{-1}$. Toutefois, du fait de la sensibilité importante qu'a montrée *T. suecica* à l'égard de l'Ag et du Cu, de nouvelles cultures ont été contaminées par des surcharges plus faibles (10 $\mu g\ Ag \cdot L^{-1}$ et 30 $\mu g\ Cu \cdot L^{-1}$). Les cultures témoins ont été constituées concomitamment. Donc pour *T. suecica*, T1 représente le témoin de la culture contaminée par le Cd, et T2 le témoin de celles contaminées par l'Ag ou le Cu.

La densité numérique des cellules est estimée par comptage au microscope sur lame hématimétrique de type Malassez (six replicats, sauf pour T2 *T. suecica* où $n = 5$). Les cultures sont arrêtées précisément lorsque les algues entament le tiers final de la phase exponentielle c'est-à-dire lorsque le nombre de divisions cellulaires diminue mais que la concentration en chlorophylle est toujours supérieure à celle des phéopigments.

Analyses biochimiques

La matière particulaire de deux échantillons de chaque culture a été recueillie sur filtre Whatman CF/C (porosité : 1 µm), puis conservés à –30°C. Les protéines ont été dosées suivant la méthode de Lowry et al. (1951, dans Baud et al. 1990). Les lipides ont été extraits dans un mélange de chloroforme et de méthanol (Bligh et Dyer 1959, dans Baud et al. 1990) puis dosés selon Marsh et Weinstein (1966, dans Baud et al. 1990). La concentration en sucres a été déterminée par la méthode de Dubois et al. (1956, dans Baud et al. 1990).

La chlorophylle *a* et phéophytine *a*, dont la somme représente les pigments totaux, ont été dosées suivant la technique de Lorenzen (1967, dans Baud et al. 1990).

Compartimentation métallique

Les cultures en grand volume ont été centrifugées. Des échantillons de 1 à 1,5 g de poids frais d'algues sont rincés pendant 10 min avec 10 mL d'un agent complexant 8-HQ (8-hydroxyquinoline-5-sulfonate, 1 mM) qui permet la désorption des métaux faiblement liés à la surface des cellules (Price et Morel 1990). Les différents échantillons sont ensuite centrifugés. Des aliquotes d'environ 100 à 400 mg de poids frais, selon l'espèce algale, ont été homogénéisées dans 2 à 3 mL de tampon Tris 0,02 M, 0,15 M NaCl (pH 8,6). Les algues ont été ensuite soumises à une sonication de 100 W à 4°C (Sonifer-250), quatre fois 20 s avec 10 s d'intervalle. La destruction cellulaire a été vérifiée au microscope optique.

La compartimentation a été réalisée selon la figure 1.

Dosage des métaux par spectrométrie d'absorption atomique (SAA)

Des aliquotes d'algues ainsi que les différents culots (C_1 et TS) issus de la compartimentation ont été préalablement minéralisés dans 1 mL d'HNO_3 (Suprapur, Merck) pendant 1 h à 90°C. Pour les surnageants (S_1 et TR), la minéralisation a été réalisée par addition d'HNO_3 seulement.

Les concentrations métalliques ont été déterminées par spectrométrie d'absorption atomique à effet Zeeman (Hitachi Z-8100) avec la méthode des ajouts dosés. Les analyses ont été effectuées

© 1999 NRC Canada

Fig. 1. Compartimentation des métaux chez les algues.

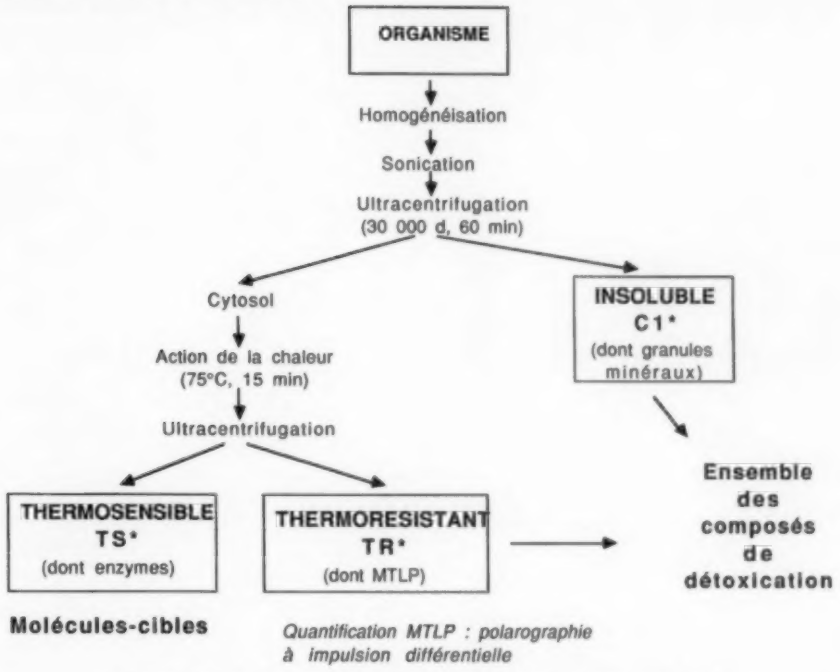
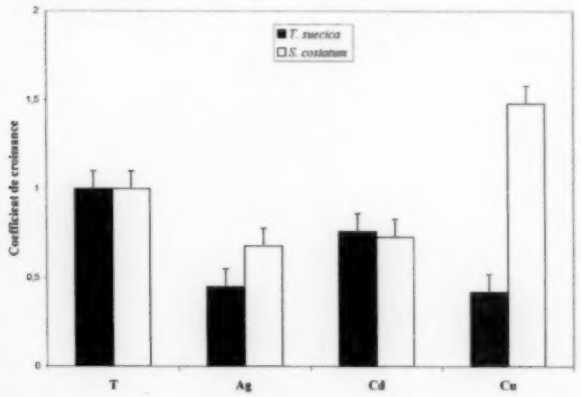


Fig. 2. Coefficient de croissance des cultures témoins et contaminées de *S. costatum* et *T. suecica* (correspond au rapport entre le nombre de cellules des cultures contaminées et celui des cultures témoins).



en four selon la méthode décrite par Amiard et al. (1987). Cette dernière a été validée par des contrôles internes et externes de qualité. Pour la première procédure, du matériel standard (tissu d'huître, SRM 1566) certifié par l'US/NBS (<<United States national Bureau of Standards>>) a été utilisé. La seconde procédure (homogénats de crevette, MA-A-3/TM) a été réalisée dans le cadre d'exercices d'intercalibrations organisés par l'IAEA (<<International Atomic Energy Agency>>).

Dans le cas de la Diatomée *S. costatum*, la présence de la silice non solubilisée par l'acide nitrique n'a pas perturbé la détermination des concentrations métalliques (Ettajani et al. 1992).

Analyses statistiques

Les résultats de la composition biochimique des algues ont été analysés à l'aide du test-*t* de Student.

Résultats

Effets sur la croissance et la composition biochimique des algues

Les effets de l'Ag, du Cd et du Cu sur la croissance des deux microalgues *S. costatum* et *T. suecica* exprimés par le coefficient de croissance (rapport entre le nombre de cellules des cultures contaminées et celui des cultures témoins) sont représentés dans la figure 2.

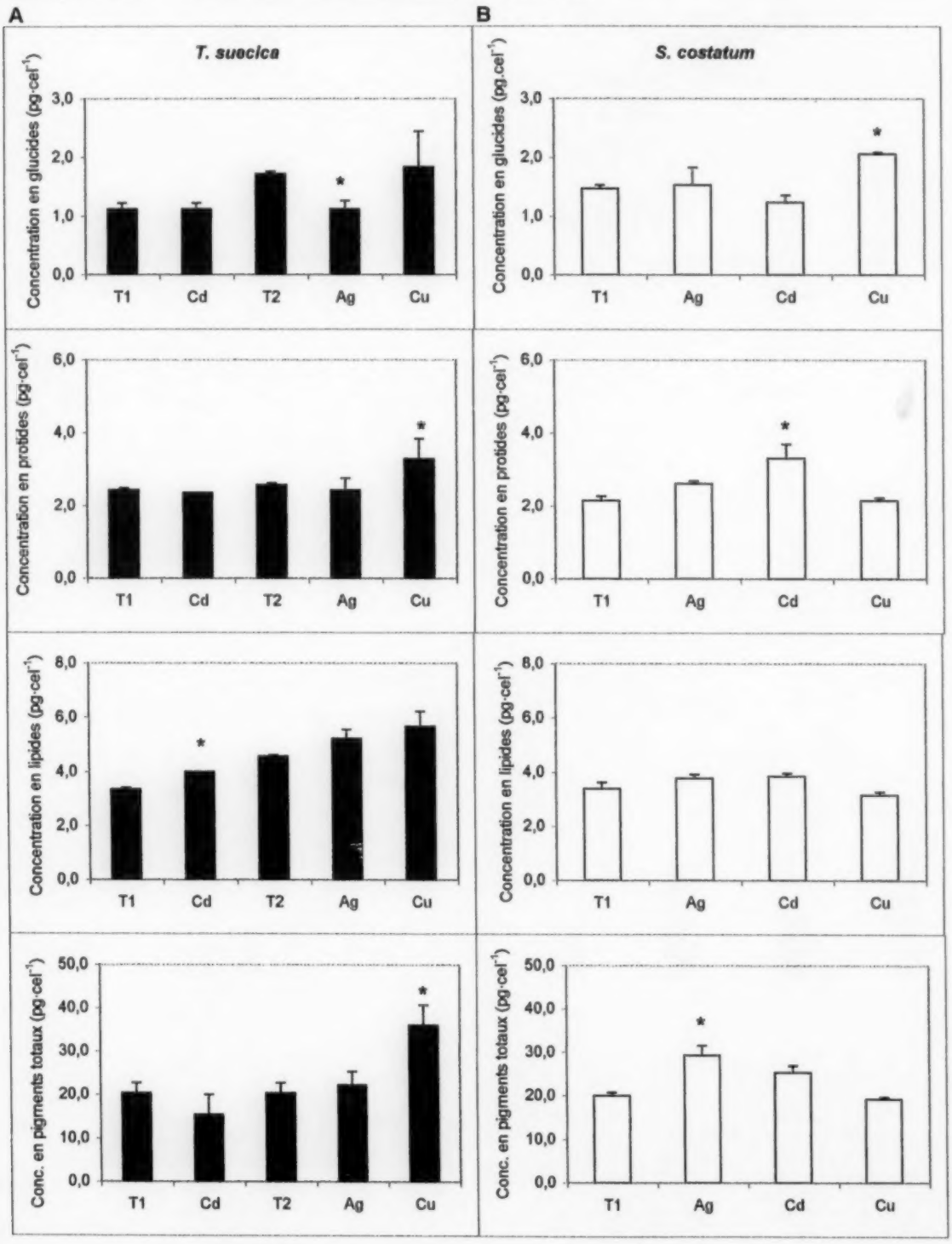
À l'arrêt des cultures des témoins (T1 et T2) de *T. suecica*, la différence du nombre de cellules est non significative (T1 : $x = 371 \times 10^3$ cellules/mL, pour $n = 6$ et T2 : $x = 390 \times 10^3$ cellules/mL, pour $n = 5$).

En présence de l'Ag, le coefficient de croissance des algues diminue considérablement aussi bien pour *S. costatum* (32%) que pour *T. suecica* (55%). En effet, pour *S. costatum* contaminée par l'Ag, la diminution de la biomasse est significative par rapport au témoin ($p < 0,05$), alors que pour le Cd, elle est non significative ($p > 0,05$). Pour *T. suecica* contaminée par l'Ag ou par le Cd, la diminution de la biomasse par rapport au témoin est hautement significative ($p < 0,01$). Toutefois, pour le Cu, les deux espèces réagissent différemment : *T. suecica*, plus sensible, a montré une croissance limitée pour la surcharge de 30 µg $Cu \cdot L^{-1}$, alors que la production de *S. costatum* a augmenté pour une concentration plus élevée de 50 µg $Cu \cdot L^{-1}$.

Les résultats des analyses biochimiques au niveau des deux algues sont représentées dans la figure 3.

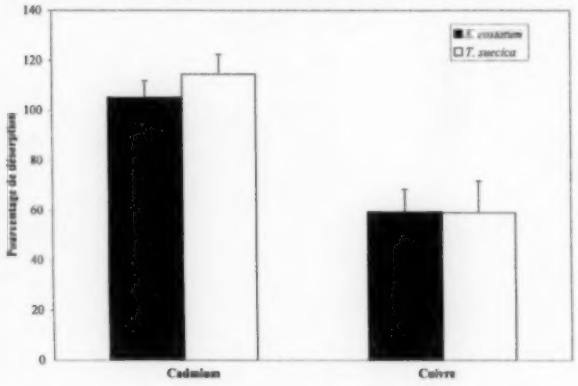
© 1999 NRC Canada

Fig. 3. Effets des métaux sur la concentration cellulaire en glucides, protides, lipides et pigments totaux chez (A) *T. suecica* et (B) *S. costatum* (concentration moyenne en pg/cellule (pg/cel) affectée de l'écart-type et $n = 2$, avec T1 : témoin relatif à la culture contaminée par le Cd et T2 : témoin relatif à celles contaminées par l'Ag ou le Cu).



© 1999 NRC Canada

Fig. 4. Pourcentage de désorption du Cd et du Cu chez *S. costatum* et *T. suecica* induit par l'agent complexant (8-HQ) (résultats exprimés par rapport à une quantité métallique initiale de 100% chez les algues avant le traitement par le 8-HQ, $n = 2$).



Chez *T. suecica*, à l'exception des cultures contaminées par l'Ag qui a provoqué une diminution significative ($p < 0,05$) des teneurs glucidiques, les autres contaminants n'ont eu aucun effet. L'augmentation enregistrée pour la culture contaminée par le Cu s'avère non significative ($p = 0,403$). Pour *S. costatum*, le Cu a eu un effet positif significatif ($p < 0,01$).

Les concentrations protidiques chez *T. suecica* contaminée par le Cu sont significativement plus élevées que celles des témoins ($p < 0,01$). Il en est de même pour *S. costatum* contaminée par le Cd ($p = 0,051$).

Pour les lipides, toutes les cultures contaminées de *T. suecica* présentent des teneurs supérieures aux témoins mais non significatives sauf pour le Cd ($p < 0,01$), alors qu'aucune influence significative des métaux n'a été constatée chez *S. costatum*.

Les teneurs en pigments totaux ont augmenté chez *T. suecica* contaminée par le Cu ($p < 0,05$) et chez *S. costatum* contaminée par l'Ag ($p < 0,05$).

Ces résultats montrent que les surcharges métalliques choisies ont provoqué une diminution non négligeable de la biomasse sans effet négatif sur la composition biochimique des deux microalgues.

Accumulation métallique

L'action de l'agent complexant (fig. 4) a entraîné le relargage d'une part importante de Cu (40%) aussi bien pour *S. costatum* que pour *T. suecica*, alors qu'aucune désorption n'a eu lieu pour celles contaminées par le Cd. La dispersion importante des résultats relatifs au dosage de l'Ag nous a interdit toute interprétation. Des interactions de l'Ag avec la solution de désorption ont éventuellement pu se produire donnant lieu à des phénomènes de relargage très aléatoires.

Après rinçage des algues à l'agent complexant, seule *S. costatum* contaminée par le Cu a présenté une légère perte en poids sec (12%).

Les concentrations moyennes d'Ag, de Cd et de Cu des algues témoins et contaminées sont présentées dans le tableau 1. Les surcharges d'Ag et de Cu introduites dans les milieux de culture des deux espèces n'étant pas les mêmes, le facteur de concentration (F_c) a été calculé. Celui-ci correspond au rapport entre la concentration du métal dans l'algue et celle dans l'eau contaminée. Selon l'espèce, l'accumulation des métaux est différente, *T. suecica* présente des facteurs de concentrations plus élevés.

Tableau 1. Concentrations métalliques moyennes (affectées de l'écart-type, $n = 3$) et facteurs de concentration (F_c) de *S. costatum* et *T. suecica* témoins et contaminées par l'Ag, le Cd, ou le Cu.

	Concentrations métalliques ($\mu g \cdot g^{-1}$ poids sec)		
Algues	Ag	Cd	Cu
	Algues témoins		
S. costatum	0,010 (0,002)	0,03 (0,01)	1,33 (0,06)
T. suecica	0,031 (0,004)	1,8 (0,3)	7,7 (0,2)
	Algues contaminées		
S. costatum	1,52 (0,05)	51,9 (2,0)	169,0 (2,6)
T. suecica	1,30 (0,22)	196,5 (7,8)	217,0 (3,9)
	Facteur de concentration (F_c)		
S. costatum	76	2600	3380
T. suecica	130	9825	7233

Compartimentation métallique

La répartition des métaux entre les différents compartiments est identique chez les deux espèces (fig. 5). Les métaux sont principalement associés à la fraction insoluble. Au sein du cytosol, la fraction thermorésistante prédomine à l'exception de l'Ag chez *T. suecica*.

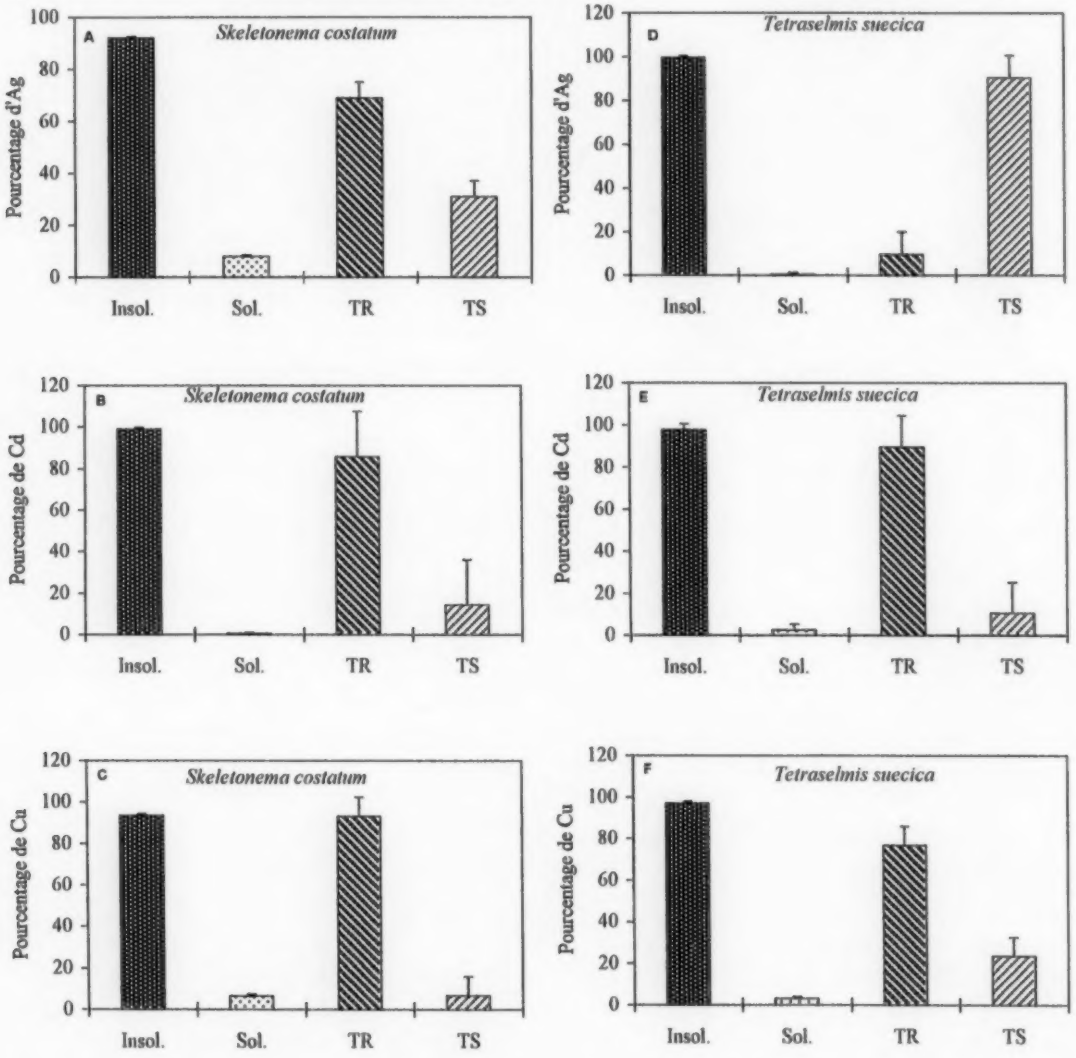
Discussion et conclusions

L'agent complexant a eu des conséquences minimes sur le poids sec des algues, ce qui montre sa spécificité pour ne désorber que les métaux faiblement fixés aux cellules, et la possibilité de l'utiliser tout en conservant l'intégrité de ces dernières. En effet, l'action de l'agent complexant montre qu'une part importante du Cu est faiblement adsorbée au niveau de la paroi algale. Ce Cu se trouve lié aux protéines carboxyliques et aux résidus aminés (Stauber et Florence 1987). Ces mêmes auteurs (Stauber et Florence 1990) ont montré que le Zn est majoritairement adsorbé à la surface des cellules, seuls 3 à 4% du Zn extracellulaire traverse la membrane. Ceci montre la biodisponibilité potentielle de ces éléments pour les maillons suivants. Quant au Cd, il serait soit fortement fixé à la surface des cellules, soit nullement fixé à ces dernières. La prise du Cd par les cellules suit deux phases : une phase rapide avec fixation du métal à la surface des algues, et une phase lente avec le passage et le transfert du métal à l'intérieur de la cellule (Roméo et Gnassia-Barelli 1985; Morelli et Scarano 1995).

D'un point de vue méthodologique, l'utilisation de l'agent complexant 8-HQ permettrait pour les études de compartimentation, d'éliminer la fraction métallique faiblement liée à la membrane algale qui risque, après broyage des échantillons dans le tampon, de se distribuer aléatoirement entre les deux fractions (soluble et insoluble) compliquant de ce fait l'interprétation des résultats. De plus, il permettrait également d'évaluer la part des métaux liés aux groupements membranaires (Morelli et Scarano 1995).

© 1999 NRC Canada

Fig. 5. Distribution de l'Ag, du Cd et du Cu dans les cellules de *S. costatum* (A, B et C) et *T. suecica* (D, E et F). Y, quantité des métaux sous forme soluble (Sol.) et insoluble (Insol.) en pourcentages (%) de la concentration globale dans les algues et quantité des métaux liés à des composés thermorésistants (TR) ou thermosensibles (TS) en pourcentages (%) de la concentration globale cytosolique (Sol.) (moyennes et intervalles de confiance, $p < 0,05$, $n = 3$).



L'adsorption des métaux à la surface algale et leur absorption dépendent du métal d'une part, et de sa forme physico-chimique d'autre part. En effet, Mason et Jenkins (1995) dans leur synthèse bibliographique distinguent les métaux de classe A (Al) de ceux de classe B (Ag) ou de transition (Cd et Cu). Les premiers se lient principalement aux ligands de l'oxygène et par conséquent n'ont pas de porteurs protéiniques spécifiques pour leur transport de la surface cellulaire au cytoplasme. Les seconds, ayant une grande affinité pour les sulfures, se lient plutôt aux protéines membranaires avant d'être transportés au niveau du cytoplasme. Par ailleurs, certains métaux se fixent aux hydroxydes de Fe ou de Mn recouvrant la membrane, ce qui peut constituer un moyen de protection de la cellule vis-à-vis de ces métaux.

En ce qui concerne la spéciation métallique, son influence sur l'absorption des métaux a été largement étudiée, et il est bien établi que les complexes métalliques liposolubles et les formes ioniques ou faiblement complexées sont plus biodisponibles (Stauber et Florence 1987; Lage et al. 1996). Les processus de transfert sont toutefois complètement différents; les premiers pénétrant dans la cellule principalement par diffusion passive, les autres donnant lieu à des phénomènes de transport actif ou facilité (Morrison 1989).

Dans le cas de notre étude, les métaux sont introduits individuellement sous forme ionique. Toutefois, au cours du développement algal, la phase aqueuse s'enrichit en diverses substances organiques excrétées par les cellules. Ce phénomène peut conduire à une modification non négligeable de la

© 1999 NRC Canada

capacité complexante du milieu vis-à-vis des métaux comme le cuivre, réputé pour former des complexes stables avec divers ligands organiques.

De plus, dans le milieu naturel les microalgues sont rarement exposées à un seul métal. Des interactions entre les métaux peuvent conduire à une exaltation (synergie entre Se et Cd) (Boisson et al. 1989) ou à une limitation (antagonisme entre Ag et Cu) (Ettajani et Pirastru 1992) de leurs effets toxiques individuels. Ces compétitions peuvent avoir lieu aussi bien au niveau des ligands membranaires qu'intracellulaires (Mason et Jenkins 1995).

En terme de bioaccumulation, une différence importante entre les deux espèces a été notée. *Tetraselmis suecica* accumulant plus d'éléments, alors même que les surcharges d'Ag et de Cu introduites dans son milieu de culture sont relativement plus faibles. En effet, *S. costatum* est une algue connue pour excréter d'assez grandes quantités de matière organique (Berland et al. 1977), celle-ci en complexant les métaux du milieu réduirait la concentration disponible et par conséquent la toxicité de ces éléments pour les algues (Brown et al. 1988). De plus, cette différence d'accumulation n'est pas sans conséquence sur le transfert des métaux des deux algues au maillon suivant (H. Ettajani, B. Berthet, J.C. Amiard et L. Chevolot, étude soumise).

Les surcharges métalliques choisies ont eu un rôle inhibiteur non négligeable sur la croissance des deux espèces d'algues, alors qu'aucune diminution significative des constituants cellulaires (glucides, protides, lipides, chlorophylle *a* et pigments totaux) n'a été notée, à l'exception des glucides chez *T. suecica* contaminée par l'Ag. Ballan-Dufrançais et al. (1991) ont montré que l'Ag peut induire des perturbations au niveau du système enzymatique de stockage des polysaccharides. Quant aux concentrations de protides, lipides et pigments totaux, ils ont connu dans la majorité des cas une augmentation significative selon l'espèce et le métal. En effet le Cu, élément essentiel, a conduit à une augmentation de ces composants, surtout chez *T. suecica*, alors même qu'en terme de croissance, cette espèce semblait plus sensible à cet élément. Quant au Cd, élément n'ayant aucun rôle physiologique connu actuellement, un effet positif a été noté au niveau des lipides et des protides respectivement chez *T. suecica* et *S. costatum*.

Plusieurs travaux soulignent la grande sensibilité de la croissance vis-à-vis des polluants par rapport aux autres processus physiologiques : photosynthèse, respiration, absorption du phosphate et de l'azote (Berland et al. 1977; Stauber et Florence 1987; Nalewajko et Olaveson 1994). L'effet toxique du Cu chez *Nitzschia closterium* se manifeste au niveau du cytosol par la diminution de la concentration intracellulaire en thiols, nécessaire à la multiplication cellulaire, ce qui entraîne une inhibition de celle-ci sans pour cela affecter les autres fonctions (Stauber et Florence 1987).

L'augmentation des produits de stockage tels que les lipides et les polysaccharides chez *S. costatum*, serait le résultat d'un vieillissement de la population, l'élément toxique inhibant ou perturbant sélectivement la pénétration des éléments essentiels et leur assimilation par la cellule (Berland et al. 1977), comme le phosphate et l'azote (Nalewajko et Olaveson 1994). Pour d'autres espèces telles que *Selenastrum capricornutum*, cette augmentation peut être une réponse d'acclimatation vis-à-vis des polluants comme le Cd (Thompson et Couture 1990).

Quant à l'augmentation des concentrations protidiques chez les deux algues contaminées par le Cd, elle représenterait une synthèse de novo de protéines pour lier cet élément (Thompson et Couture 1990). De plus, Price et Morel (1990) soulignent le rôle positif que peut jouer le Cd dans les milieux déficients en Zn. En effet, le Cd est susceptible de remplacer cet élément essentiel au niveau des macromolécules favorisant de ce fait la croissance des diatomées marines.

Le faible effet des différents métaux étudiés sur la composition biochimique des micro-algues est à relier également aux résultats relatifs à la compartimentation. En effet, les métaux se trouvant majoritairement dans la phase insoluble, une part importante serait liée à la membrane cellulaire ou encore détoxifiée chez *T. suecica* au niveau des vésicules osmiophiles intracytoplasmiques (Ballan-Dufrançais et al. 1991). Chez la diatomée *Thalassiosira weissflogii*, une part importante de l'Ag, du Cd et du Zn est associée à la fraction insoluble rendant ces derniers moins disponibles aux consommateurs primaires (Price et Morel 1990; Connel et al. 1991).

L'association plus marquée de l'Ag aux composés thermosensibles (TS) du cytosol chez *T. suecica* suggère une sensibilité plus grande de cette espèce par comparaison à *S. costatum*. En effet, cette fraction contient la majorité des enzymes qui constituent la cible moléculaire des métaux toxiques.

La tolérance différentielle des deux microalgues rencontrée dans cette étude corrobore les résultats de travaux selon lesquels *S. costatum* serait plus résistante que d'autres microalgues, surtout celles n'appartenant pas aux diatomées, à l'Ag et à l'As (Sanders et Cibik 1988; Sanders et Abbe 1989), au Cu (Metaxas et Lewis 1991) et au Zn (Smith 1983). De plus, les deux espèces montrent des profils chromatographiques bien distincts suggérant la présence de composés fixant les métaux de poids moléculaire apparent différent. Ni la caractérisation par HPLC, ni l'analyse des spectres UV de ces composés n'ont pu affirmer l'existance de phytochélatines dans la phase soluble de ces deux espèces (H. Ettajani, B. Berthet, J.C. Amiard et L. Chevolot, étude soumise). Ils constituent, chez les plantes et les algues, les principaux chélateurs intracellulaires des métaux essentiels ou non essentiels; le Cd représentant l'élément le plus inducteur, suivi de l'Ag et du Cu (Grill et al. 1987). Contrairement à la métallothionéine, ces peptides ((-Glu-Cys)n-Gly avec n = 2–11)) ne sont pas induits directement par les gènes, mais résultent de réactions enzymatiques impliquant la phytochélatine-synthase (Rauser 1990). La synthèse de tels composés dépend des espèces algales et des concentrations métalliques choisies (Rauser 1990; Rijstenbil et al. 1994). En effet, dans la majorité des cas, la présence de ces peptides a été établie surtout chez les plantes, naturellement soumises à de fortes concentrations des métaux dans le sol (Grill et al. 1987), sinon chez des algues mais sous des conditions de contamination relativement élevées, de l'ordre de 1 mg Cd·L^{-1} pour *Chlorella ellipsoidea* (Nagano et al. 1984) et *Phaeodactylum tricornutum* (Kawaguchi et Maita 1990), à l'exception de l'étude de Ahner et al. (1994) concernant la production de ces composés chez des algues sous des conditions de faibles contaminations métalliques.

© 1999 NRC Canada

Dans le cas de cette étude, où les concentrations métalliques choisies sont réalistes par rapport à celles rencontrées dans des milieux « naturels » pollués, il apparaît donc que les composés types phytochélatines sont peu ou pas sollicités par les deux espèces *S. costatum* et *T. suecica*. Par ailleurs, compte tenu des fortes concentrations des trois métaux dans la fraction insoluble, la formation de minéraux et (ou) la fixation des métaux au niveau de la membrane permet d'éviter ou de réduire la toxicité de ces éléments. En effet, chez *Chlamydomonas bullosa* contaminée par le Cu, le développement d'organites membranaires constitue un mécanisme majeur de détoxication (Visviki et Rachlin 1994). Néanmoins, en dépit de ces moyens, l'effet des trois métaux étudiés (Ag, Cd et Cu) sur la croissance algale est non négligeable (à l'exception du Cu chez *S. costatum*). Il est possible que (*i*) par leur liaison à des composés membranaires, ces métaux provoqueraient une compétition pour l'absorption d'éléments nécessaires à la multiplication cellulaire comme le fer, le zinc, etc. (Morel et Hudson 1991) ou encore (*ii*) les métaux auraient comme première cible les paramètres liés à la division cellulaire tels que la concentration intracellulaire en thiols qui, en diminuant, entraîne une baisse de la défense de la cellule vis-à-vis du H_2O_2 (Stauber et Florence 1987).

La réduction de la croissance des algues, soumises même à de faibles concentrations métalliques, constitue une des premières manifestations de la toxicité de ces éléments. Toutefois, le faible effet sur la composition biochimique permet de préserver la valeur nutritive de ces algues.

En terme de transfert métallique, la liaison importante des métaux aux composés insolubles d'une grande stabilité, suggère un faible transfert aux consommateurs. Toutefois, une telle hypothèse reste à confirmer par des études simulant l'absorption digestive chez ces derniers (attaque enzymatique et (ou) action du pH) des métaux bioaccumulés par les algues.

Remerciements

Nous remercions J.P. Baud (Institut français de recherche pour l'exploitation de la mer, Bouin), J.M. Robert (Laboratoire de Biologie Marine, Université de Nantes) et leurs équipes, particulièrement Y. Nassiri, pour l'aide qu'ils nous ont apportée dans ce travail à des titres divers.

Bibliographie

Ahner, B.A., Price, N.M., et Morel, F.M.M. 1994. Phytochelatin production by marine phytoplankton at low free metal ion concentrations : laboratory studies and field data from Massachusetts Bay. Proc. Natl. Acad. Sci. U.S.A. **91** : 8433–8436.

Amiard, J.-C., Pineau, A., Boiteau, H.L., Métayer, C., et Amiard-Triquet, C. 1987. Application de la spectrométrie d'absorption atomique Zeeman aux dosages de huit éléments traces (Ag, Cd, Cr, Cu, Mn, Ni, Pb et Se) dans des matrices biologiques solides. Water Res. **21** : 693–697.

Amiard, J.C., Amiard-Triquet, C., Ballan-Dufrançais, C., Berthet, B., Jeantet, A.Y., Martoja, R., et Truchet, M. 1989. Study of the bioaccumulation at the molecular, cellular and organism levels of lead and copper transferred to the oyster *Crassostrea gigas* Thunberg directly from water or *via* food. Comptes rendus de la 20e EMBS (<<European Marine Biology Symposium>>), Gdansk, du 14 au 19 septembre 1986. Polish. Acad. Sci. Inst. Oceanology, Warsaw. pp. 521–529.

Amiard-Triquet, C., Martoja, R., et Marcaillou, C. 1992. Alternative methodologies for predicting metal transfer in marine food webs including filter-feeders. Water Sci. Technol. **25** : 197–204.

Ballan-Dufrançais, C., Marcaillou, C., et Amiard-Triquet, C. 1991. Response of the phytoplanktonic alga *Tetraselmis suecica* to copper and silver exposure : vesicular metal bioaccumulation and lack of starch bodies. Biol. Cell, **72** : 103–112.

Baud, J.P., Haure, J., et Garnier, J. 1990. Effets de l'apport de phytoplancton (*Skeletonema costatum*) sur la croissance et les variations de la composition biochimique de la palourde japonaise *Ruditapes philippinarum* cultivée en marais. Océanis, **16** : 391–408.

Berland, B.R., Bonin, D.J., Guérin-Ancey, O.J., Kapkov, V.I., et Arlhac, D.P. 1977. Action de métaux lourds à des doses sublétales sur les caractéristiques de la croissance chez la diatomée *Skeletonema costatum*. Mar. Biol. **42** : 17–30.

Boisson, F., Gnassia-Barelli, M., Chiavereni, J., et Roméo, M. 1989. Effect of selenium on the uptake of cadmium by the marine microalga *Hymenomonas (Cricosphaera) elongata*. Mar. Environ. Res. **28** : 465–469.

Brown, L.N., Robinson, M.G., et Hall, B.D. 1988. Mechanisms for copper tolerance in *Amphora coffeaeformis* : internal and external binding. Mar. Biol. **97** : 581–586.

Connel, D.B., Sanders, J.G., Riedel, GF., et Abbe, G. 1991. Pathways of silver uptake and trophic transfer in estuarine organisms. Environ. Sci. Technol. **25** : 921–924.

Ettajani, H., et Pirastru, L. 1992. Méthodologie pour prévoir le transfert des métaux lourds dans les chaînes trophiques marines incluant des Mollusques filtreurs. Hydroécol. Appl. **4** : 79–90.

Ettajani, H., Amiard-Triquet, C., et Amiard, J.C. 1992. Étude expérimentale du transfert de deux éléments traces (Ag, Cu) dans une chaîne trophique marine : eau – particules (sédiment naturel, microalgue) – mollusques filtreurs (*Crassostrea gigas* Thunberg). Water Air Soil Pollut. **65** : 215–236.

Grill, E., Winnacker, E.-L., et Zenk, M.H. 1987. Phytochelatins, a class of heavy-binding peptides from plants, are functionally analogous to metallothioneins. Proc. Natl. Acad. Sci. U.S.A. **84** : 439–443.

Kawaguchi, S., et Maita, Y. 1990. Amino acid sequence of cadmium-binding peptide induced in a marine diatom, *Phaeodactylum tricornutum*. Bull. Environ. Contam. Toxicol. **45** : 893–899.

Lage, O.M., Soares, H.M.V.M., Vasconcelos, M.T.S.D., Parent, A.M., et Salema, R. 1996. Toxicity effects of copper (II) on the marine dinoflagellate *Amphidinium carterae* : influence of metal speciation. Eur. J. Phycol. **31** : 341–348.

Mason, A.Z. et Jenkins, K.D. 1995. Metal speciation and bioavailability in aquatic systems. *Dans* IUPAC, Series on Analytical and Physical Chemistry of Environmental Systems. Vol. 3. *Éditeurs* : A. Tessier et D.R. Turner. John Wiley and Sons, Chicester. pp. 479–608.

Metaxas, A., et Lewis, A.G. 1991. Copper tolerance of *Skeletonema costatum* and *Nitzschia thermalis*. Aquat. Toxicol. **19** : 265–280.

Morel, F.M.M., et Hudson, R.J.M. 1991. Limitation of productivity by trace metals in the sea. Limnol. Oceanogr. **36** : 1742–1755.

Morelli, E., et Scarano, G. 1995. Cadmium induced phytochelatins in marine alga *Phaeodactylum tricornutum* : effect of metal speciation. Chem. Spec. Bioavail. **7** : 43–47.

Morrison, G.M.P. 1989. Trace element speciation and its relationship to bioavailability and toxicity in natural waters. *Dans* Trace

© 1999 NRC Canada

element speciation analytical methods and problems. *Éditeur* : G.E. Batley. CRC Press, Boca Raton. pp. 25–41.

Nagano, T., Miwa, M., Suketa Y., et Okada, S. 1984. Isolation, physicochemical properties, and amino acid composition of a cadmium-binding protein from cadmium-treated *Chlorella ellipsoidea*. J. Inorg. Biochem. **21** : 61–71.

Nalewajko, C., et Olaveson, M.M. 1994. Differential responses of growth, photosynthesis, respiration, and phosphate uptake to copper in copper-tolerant and copper-intolerant strains of *Scenedesmus acutus* (Chlorophyceae). Can. J. Bot. **73** : 1295–1303.

Nyholm, N., et Damgaard, B.M. 1990. A comparison of the algal growth inhibition toxicity test method with the short term ^{14}C-assimilation test. Chemosphere, **21** : 671–679.

Price, N.M., et Morel, F.M.M. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. Nature (London), **344** : 658–660.

Rauser, W.E. 1990. Phytochelatins. Annu. Rev. Biochem. **59** : 61–86.

Rijstenbil, J.W., Sandee, A., Drie, J.V., et Wijnhoids, J.A. 1994. Interaction of toxic trace metals and mechanisms of detoxification in the planktonic diatoms *Ditylum brightwellii* and *Thalassiosira pseudonana*. FEMS Microbiol. Rev. **14** : 387–396.

RNO (Réseau national d'observation). 1995. Surveillance du milieu marin. Ministère de l'environnement. Édition 1995. Institut français de recherche pour l'exploitation de la mer, France.

Roméo, M., et Gnassia-Barelli, M. 1985. Metal uptake by different species of phytoplankton in culture. Hydrobiologia, **123** : 205–209.

Sanders, J.G., et Abbe, G.R. 1989. Silver transport and impact in estuarine and marine systems. *Dans* Aquatic toxicology and environmental fate. Vol. 11. *Éditeurs* : G.W. Suter II et M.A. Lewis. American Society for testing and materials, Philadelphia, Pa. pp. 5–18.

Sanders, J.G., et Cibik, S.J. 1988. Response of Chesapeake Bay phytoplankton communities to low levels of toxic substances. Mar. Pollut. Bull. **19** : 439–444.

Smith, M.A. 1983. The effects of heavy metals on the cytoplasmic fine structure of *Skeletonema costatum* (Bacillariophyta). Protoplasma, **116** : 14–23.

Stauber, J.L., et Florence, T.M. 1987. Mechanism of toxicity of ionic copper and copper complexes to algae. Mar. Biol. **94** : 511–519.

Stauber, J.L., et Florence, T.M. 1990. Mechanism of toxicity of zinc to marine diatom *Nitzschia closterium*. Mar. Biol. **105** : 519–524.

Thompson, P.A., et Couture, P. 1990. Aspects of carbon metabolism in the recovery of *Selenastrum capricornutum* populations exposed to cadmium. Aquat. Toxicol. **17** : 1–14.

Visviki, I., et Rachlin, J.W. 1992. Ultrastructural changes in *Dunaliella minuta* following acute and chronic exposure to copper and cadmium. Arch. Environ. Contam. Toxicol. **23** : 420–425.

Visviki, I., et Rachlin, J.W. 1994. Acute and chronic exposure of *Dunaliella salina* and *Chlamydomonas bullosa* to copper and cadmium : effects on growth. Arch. Environ. Contam. Toxicol. **26** : 149–153.

© 1999 NRC Canada

Phylogeographic lineages and differentiation pattern of Atlantic salmon (*Salmo salar*) in the Baltic Sea with management implications

Marja-Liisa Koljonen, Håkan Jansson, Tiit Paaver, Oleg Vasin, and Jarmo Koskiniemi

Abstract: The genetic structure and isolation pattern of the Atlantic salmon (*Salmo salar*) throughout its range in the Baltic Sea were examined as a starting point for a conservation strategy for the species in this area. The allozyme variation in seven polymorphic loci was studied in 5125 salmon from 24 rivers in four countries. A clear dichotomy was observed between stock groups from southeastern (Russia, Estonia, Latvia, southern Sweden) and northwestern (northern Finland, northern Sweden) drainage regions, corresponding to the postglacial colonisation of the Baltic Sea by two phylogeographic lineages, one from the east (the Ice Lake lineage) and one from the west (the Atlantic lineage). The geographical and genetic distances between stocks fit the one-dimensional "isolation-by-distance" model ($p < 0.001$). The estimated gene flow ranged from 0 to10 migrants per generation. The total diversity of hatchery stocks was 72% of that of the wild stocks. Genetically similar stock groups, phylogeographic lineages, and drainage regions are recommended for use as genetic management units in addition to stock level.

Résumé : On a examiné la structure génétique et le profil d'isolement du saumon atlantique (*Salmo salar* L.) dans toute son aire de répartition dans la mer Baltique en vue d'établir une stratégie de conservation de l'espèce dans cette région. On a étudié la variation des allozymes de sept loci polymorphes chez 5125 saumons prélevés dans 24 rivières de quatre pays. On a observé une nette dichotomie entre les groupes de stocks des bassins hydrographiques du sud-est (Russie, Estonie, Lettonie, sud de la Suède) et des bassins du nord-ouest (nord de la Finlande, nord de la Suède), qui s'explique par la colonisation postglaciaire de la mer Baltique par deux lignées phylogéographiques, l'une de l'est (lignée du lac glaciaire) et l'une de l'ouest (lignée de l'Atlantique). Les distances géographiques et génétiques entre les stocks s'accordent avec le modèle unidimensionnel d'isolement en fonction de la distance ($p < 0{,}001$). Le flux génétique estimé variait de 0 à 10 migrants par génération. La diversité totale des stocks d'écloserie était de 72 % de celle des stocks sauvages. En plus de la taille des stocks, on recommande de prendre en considération dans les unités génétiques de gestion les groupes de stocks génétiquement similaires, les lignées phylogéographiques et les bassins versants.

[Traduit par la Rédaction]

Introduction

A hierarchical structure of population differentiation is evident among Atlantic salmon (*Salmo salar*) populations throughout the range of the species. Baltic salmon constitute one of the three major groups of the species, the others being the west and east Atlantic groups (Ståhl 1987) or races (Cross et al. 1998). Nowadays, Baltic salmon as a whole form one effectively isolated evolutionary unit of Atlantic salmon and differ clearly from Atlantic salmon of the rivers draining into the Atlantic Ocean and Barents Sea (Ståhl 1987; Koljonen 1989; Kazakov and Titov 1993). A study on Baltic and North Sea populations showed that more than half of the total between-population component (57.5%) derived from a major branching between Atlantic and Baltic areas (Ryman 1983).

The status of natural populations in the Baltic Sea is threatened and their preservation for the future can only be assured by fishing regulation and other conservation measures. The primary reason for loss of the bulk of Baltic salmon stocks is destruction of their reproduction habitats by powerplant construction. Further threats are posed by overfishing in relation to the natural production level and, since 1992, by the highly increased incidence of yolk-sac mortality due to the M-74 syndrome. The majority of smolts (90%) are currently produced in hatcheries. Baltic salmon stocks mix offshore during their feeding and spawning migration and are a target of the intensive international mixed-stock fishery. It is therefore important to include all salmon stocks throughout the Baltic region in analyses. Only then can we

Received September 22, 1998. Accepted June 8, 1999.
J14793

M.-L. Koljonen[1] **and J. Koskiniemi.** Finnish Game and Fisheries Research Institute, P.O. Box 6, FIN-00721 Helsinki, Finland.
H. Jansson. Salmon Research Institute, Forskarstigen, S-814 94 Älvkarleby, Sweden.
T. Paaver. Department of Fish Farming, Estonian Agricultural University, EE-2400 Tartu, Estonia.
O. Vasin. Latvian Fisheries Research Institute, Daugavagrivas 6, LV-1007 Riga, Latvia.

[1]Author to whom all correspondence should be addressed.
e-mail: marja-liisa.koljonen@rktl.fi

© 1999 NRC Canada

gain insight into the total evolutionary structure and the reasons for it and thus gain the basic information needed for creating a management and conservation strategy.

The primary factors in the genetic structure of Baltic salmon are related to glaciation history, to the formation of the Baltic Sea, and to the colonisation sources and directions of the salmon stocks. When the front of the ice sheet advanced from the northwest and covered all of Scandinavia, the preglacial (actually interglacial) salmon populations were forced to move southwards and into glacial ice-marginal lakes and catchments draining southwards. Glacial relicts of several species of freshwater crustaceans are thought to have immigrated into Scandinavian lakes from eastern glacial lakes rather than from a western marine Atlantic direction (Segerstråle 1976). The Atlantic salmon rivers of the Baltic Sea basin could thus also have been colonised either from the east, by refugial glacial lake populations, or from the west, by Atlantic populations in the North Sea and to the south of it, or from both sources. Since the formation of present catchments, the role of gene flow has been essential in determining the possibilities for both neutral and adaptive differentiation among salmon stocks. The most recent changes in the genetic structure of Baltic salmon have been caused by human activity.

Preserving genetic diversity implies conserving the genetic discreteness of certain defined genetic units: populations, river stocks, similarity groups, or phylogeographic lineages. The goal here has been to identify these units among Baltic salmon stocks and to show how they might serve as the building blocks of a management strategy. To clarify the reasons behind the genetic structure, the present distribution of genetic diversity in Baltic salmon stocks is attributed to three different aspects: (*i*) glacial differentiation, (*ii*) gene flow patterns in the present Baltic Sea, and (*iii*) the human impact on genetic structure. The observed differentiation pattern is evaluated in relation to the three alternative hypotheses of postglacial colonisation directions (Ice Lake, Atlantic, or both), and allele frequencies are compared with data on potential source populations outside the recent Baltic Sea. The differentiation pattern between populations is tested by the "isolation-by-distance" model, and the level of gene flow between populations is estimated. The diversity patterns of natural and hatchery stock groups and natural and hatchery strains of the Tornionjoki stock are compared. Finally, conclusions are drawn from the results for the formulation of a management and conservation strategy for Baltic salmon. This is the first time that data on eastern and western stocks have been analysed in a comparable way.

Material and methods

Stock samples

The allozyme variation of 5125 Atlantic salmon from 24 rivers or tributaries in the five countries Sweden, Finland, Russia, Estonia, and Latvia that make a major contribution to salmon production in the Baltic Sea was analysed (Table 1; Fig. 1). The stocks were divided into five groups by geographical drainage region according to the corresponding sea areas used by the International Council for the Exploration of the Sea (ICES): Bothnian Bay (31), Bothnian Sea (30), Gulf of Finland (32), Eastern Main Basin (28), and Southern Main Basin (25) (Table 1; Fig. 1). The sampled stocks constituted an overwhelming majority of wild and hatchery production, unsampled stocks accounting for only about 2%. According to an ICES report (Anonymous 1996), the total smolt production of rivers draining into the Baltic Sea was about 5 571 000 individuals in 1995, of which 5.5%, or 299 000, were naturally reproduced. Our estimate (4 862 000 smolts) is somewhat smaller than that and is based only on relatively well-known data (Table 2).

Some of the samples from Finnish and Swedish stocks have been used previously, in conjunction with mixed-stock fishing analyses (Koljonen and McKinnell 1996; Koljonen and Pella 1997) (see Table 1). New data were available for this study from four Swedish (Kalixälven, Byskeälven, Ljungan, Mörrumsån), two Finnish (Iijoki, Oulujoki), four Estonian (Kunda, Loobu, Keila, Vasalemma), and two Latvian rivers (Daugava, Venta) (Table 1). Because one of the samples, Lögdeälven, was from the published data of Ståhl (1983), and these data did not include locus *IDDH-1**, the allele frequencies of the most similar stock, Dalälven, were used instead in this locus, as was also done in an earlier study (Koljonen and Pella 1997).

The 1993 Tornionjoki hatchery sample consisted of offspring from three different broodstocks. Their proportions in the sample were weighted with the smolt production proportions in 1993. In that year-class, 50% (n = 147) of the smolts came from the Taivalkoski hatchery and 27% (n = 80) from the Lautiosaari hatchery; 23% (n = 69) were reared from wild spawners caught in the Kukkola rapids (total n = 296). The hatchery sample of 1992 was from the Taivalkoski hatchery.

Published data were used to compare the genetic distances with those of the potential source population outside the present Baltic Sea range. The eastern, refugial direction was represented by data from the Russian landlocked populations from Lakes Ladoga (Kazakov and Titov 1993) and Onega (Koljonen 1989). For these populations, the allele frequency of the most similar, Neva, stock (0.925) was used for the information lacking on the *IDDH-1** locus. The western, Atlantic direction was represented by data from a Norwegian stock from the Numedalslågen, a river draining into the North Sea (Bourke et al. 1997). Published data on the Emån, a Swedish river draining into the Western Main Basin, were also added (Ståhl 1987) (Fig. 1). For this stock, the mean of the Mörrumsån (0.991) and Dalälven (0.987) stocks was used for the information lacking on the *IDDH-1** locus. Two hatchery stocks, Finnish Oulujoki and Swedish Luleälven, were omitted from this comparison because their allele frequencies had been changed markedly by mixing of stocks.

Electrophoresis

Muscle, liver, and, occasionally, eye tissue of salmon was assayed by horizontal starch gel electrophoresis. Six enzymes coded by a total of 18 loci were analysed. Seven of the loci were variable: *sAAT-4** (aspartate aminotransferase (AAT), EC 2.6.1.1), *IDDH-1** and *IDDH-2** (L-iditol dehydrogenase (IDDH), EC 1.1.1.14), *IDHP-3** (isocitrate dehydrogenase (IDHP), EC 1.1.1.42), *sMDH-3,4** (malate dehydrogenase (MDH), EC 1.1.1.37), *mMEP-2** (malic enzyme (ME), EC 1.1.1.40), and *PGM-1** (phosphoglucomutase (PGM), EC 5.4.2.2). Electrophoresis of IDHP, MDH, MEP, and PGM was conducted with the modified buffer system (pH from 6.6 to 6.8) of Clayton and Tretiak (1972), that of AAT with the same buffer or the system of Varvio-Aho and Pamilo (1980), and that of IDDH with the buffer system of Varvio-Aho and Pamilo (1980). Staining methods were as in Koljonen and Pella (1997).

Data analysis

Several samples of each river stock were pooled in the data analyses. Heterogeneity among samples of wild and hatchery origin was observed in the Tornionjoki stock but not in the groups of the Simojoki stock, and partly for that reason, these groups were treated separately for the Tornionjoki stock, which increased the

© 1999 NRC Canada

Table 1. Composition of Atlantic salmon stock samples.

No.	Subdivision of sea	Stock	Country	Origin	Class of stock	Year	Sample *n*	Total *n*
1	31	Tornionjoki	Finland, Sweden	Wild	W	1988[a]	19	
				Wild		1988[a]	100	
				Wild		1989[a]	245	
				Wild		1989[a]	46	
				Wild		1993[b]	236	
				Hatchery		1992[b]	260	
				Hatchery		1993[b]	296	1202
2	31	Simojoki	Finland	Hatchery	W	1992[b]	689	
				Hatchery		1993[b]	30	
				Wild		1993[b]	30	749
3	31	Iijoki	Finland	Hatchery	H	1990[b]	180	
				Hatchery		1993[b]	153	
				Hatchery		1997	60	393
4	31	Oulujoki	Finland	Hatchery	H	1992[b]	180	
				Hatchery		1997	60	240
5	31	Kalixälven	Sweden	Wild	W	1987	45	
				Wild		1988	146	
				Wild		1989	583	774
6	31	Luleälven	Sweden	Hatchery	H	1993[b]		60
7	31	Byskeälven	Sweden	Wild	W	1994		30
8	31	Skellefteälven	Sweden	Hatchery	H	1993[b]		84
9	31	Vindelälven	Sweden	Wild	W	1989[a]	50	
				Wild		1990[a]	50	100
10	31	Umeälven	Sweden	Hatchery	H	1989[a]	48	
				Hatchery		1990[a]	50	98
11	31	Lögdeälven	Sweden	Wild	W	1979[c]		69
12	30	Ångermanälven	Sweden	Hatchery	H	1993[b]		100
13	30	Indalsälven	Sweden	Hatchery	H	1993[b]		90
14	30	Ljungan	Sweden	Wild	W	1996		52
15	30	Ljusnan	Sweden	Hatchery	H	1993[b]		50
16	30	Dalälven	Sweden	Hatchery	H	1988[b]		200
17	32	Neva	Russia (Finland)	Hatchery	W	1990[b]	149	
				Hatchery		1993[b]	171	320
18	32	Kunda Estonia		Wild	W	1994	25	
				Wild		1995	34	59
19	32	Loobu	Estonia	Wild	W	1994	15	
				Wild		1995	35	50
20	32	Keila	Estonia	Wild	W	1994	30	
				Wild		1995	33	63
21	32	Vasalemma	Estonia	Wild	W	1994	7	
				Wild		1995	36	43
22	28	Daugava	Latvia	Hatchery	H	1996	100	
				Hatchery		1996	25	125
23	28	Venta	Latvia	Wild	W	1996		107
24	25	Mörrumsån	Sweden	Wild	W	1994		67
Total								5125

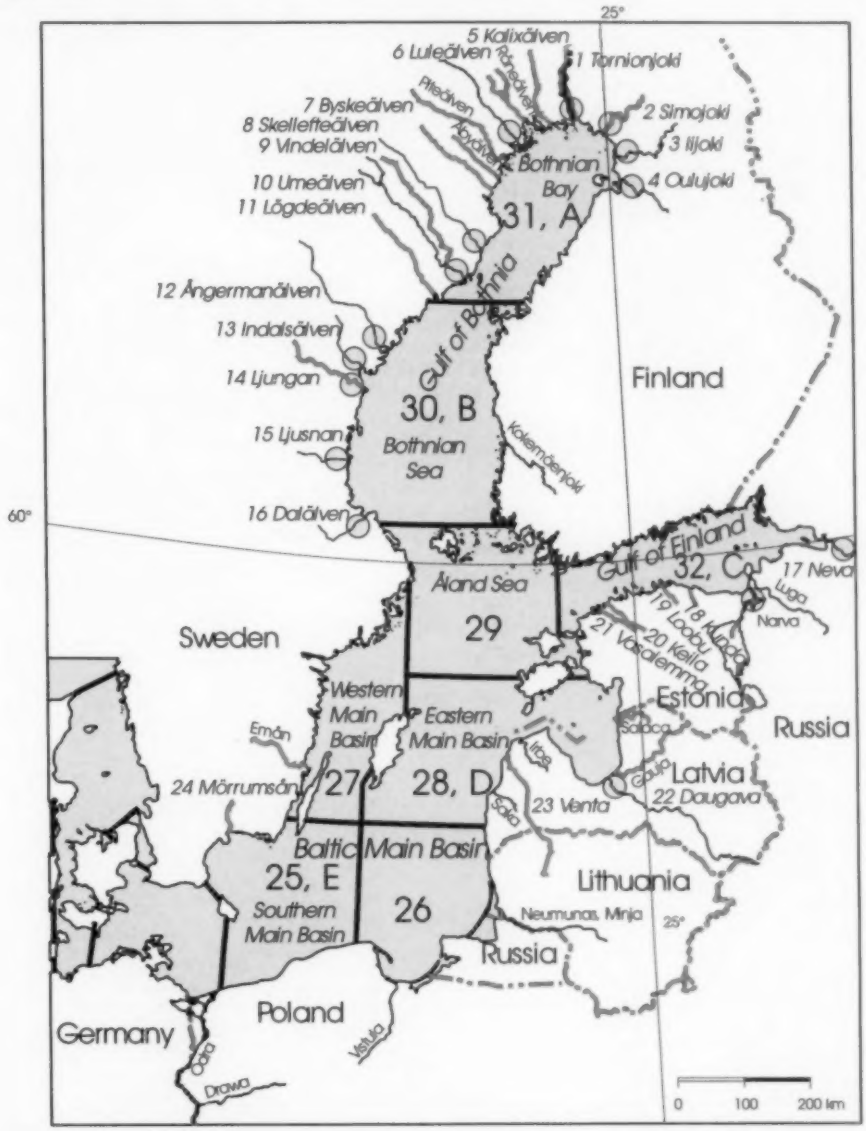
Note: The classification of each river stock is indicated by W for stocks with wild production and H for stocks with only hatchery production.
[a]Data from Koljonen and Pella (1997).
[b]Data from Koljonen and McKinnell (1996).
[c]Data from Ståhl (1983).

number of stocks studied to 25. Whenever the Hardy–Weinberg disequilibrium was observed in a river stock, each sample was submitted to further analyses.

The BIOSYS-1 program (Swofford and Selander 1981) was used to calculate allele frequencies, mean numbers of alleles per locus, and mean heterozygosities and to test the conformance of genotype frequencies with the Hardy–Weinberg equilibrium. The effect of multiple tests for the Hardy–Weinberg equilibrium was corrected with the Bonferroni procedure. The population differentiation in separate loci within different sea areas and the difference between wild and hatchery strains of the Tornionjoki stocks were tested with the exact probability test (Raymond and Rousset 1995*a*) of

© 1999 NRC Canada

Fig. 1. Map of the Baltic Sea showing ICES subdivisions 25–32. Rivers with natural Atlantic salmon production are marked in bold, and hatchery production is designated with circles at river mouths. The sea areas into which the rivers studied drain are shaded and marked with the letters A–E.



the STRUC program in the GENEPOP software package (Raymond and Rousset 1995*b*). Differentiation probabilities across loci were combined with Fisher's method. Pairwise tests for population differentiation were done by using contingency χ^2 tests.

The genetic differentiation between stocks was measured with Nei et al.'s (1983) D_A distance, which had performed well in creating the topology of the phylogenetic tree in simulation studies (Nei and Takezaki 1994; Takezaki and Nei 1996). The neighbour-joining method (Saitou and Nei 1987) was used to construct a dendrogram. The bootstrap test over loci was performed for the neighbour-joining tree by recalculating the distance for all loci 1000 times with the DISPAN package (Ota 1993).

Geographical distances between stock pairs were compared with their genetic distances to analyse the fit of the isolation-by-distance model (Wright 1943; Kimura and Weiss 1964). The significance of the fit was tested by the Mantel test of the ISOLDE program in the GENEPOP software package (version 3.1a, August 1997) (Raymond and Rousset 1995*b*) using 10 000 bootstraps. To analyse the dimensions of the isolation model, semimatrixes of weighted pairwise F_{ST} values ($F_{ST}/(1 - F_{ST})$, Weir and Cockerham 1984) and geographical distances (both distance and ln distance) between river mouths of the stocks were compared (Rousset 1997). Geographical distances were measured as straight lines over the sea between river mouths. The relationship of these two distances was plotted as log(M) against log(geographical distance) according to Slatkin (1993), where M is the estimated level of gene flow counted from the F_{ST} values (M, migrants for weighted F_{ST} values, $M = (1 - F_{ST})/(2F_{ST})$). If the data fit the isolation-by-distance model, a clear

© 1999 NRC Canada

Table 2. Estimated smolt production of natural and hatchery stocks of Atlantic salmon in the Baltic Sea in 1995 and production of each river as a proportion of the total.

Sample	Sub-division	Stock	Country	Production × 1000 fish			% of total production
				Natural	Hatchery	Total	
1	31	Tornionjoki	Finland, Sweden	75	262	337	6.9
2	31	Simojoki	Finland	1.4	154	155	3.2
3	31	Iijoki	Finland		792	792	16.3
4	31	Oulujoki	Finland		218	218	4.5
5	31	Kalixälven	Sweden	44		44	0.9
	31	Råneälven	Sweden	1.2		1.2	0.0
	31	Piteälven	Sweden	3		3	0.1
	31	Åbyälven	Sweden	1.2		1.2	0.0
6	31	Luleälven	Sweden		541	541	11.1
7	31	Byskeälven	Sweden	8.7		8.7	0.2
8	31	Skellefteälven	Sweden		122	122	2.5
9	31	Vindelälven	Sweden	15		15	0.3
10	31	Umeälven	Sweden		130	130	2.7
	31	Öreälven	Sweden	0.9		0.9	0.0
11	31	Lögdeälven	Sweden	0.9		0.9	0.0
12	30	Ångermanälven	Sweden		351	351	7.2
13	30	Indalsälven	Sweden		256	256	5.3
14	30	Ljungan	Sweden	4	30	34	0.7
15	30	Ljusnan	Sweden		231	231	4.7
16	30	Dalälven	Sweden		162	162	3.3
17	32	Neva	Russia, Finland	20	478	498	10.2
	32	Luga	Russia	10	20	30	0.6
	32	Narva	Estonia, Russia		10	10	0.2
18	32	Kunda	Estonia	1.8		1.8	0.0
19	32	Loobu	Estonia	1.8		1.8	0.0
20	32	Keila	Estonia	1.8		1.8	0.0
21	32	Vasalemma	Estonia	1.8		1.8	0.0
	28	Pärnu	Estonia	8		8	0.2
	28	Salaca	Latvia	15	94	109	2.2
	28	Gauja	Latvia	13	163	176	3.6
22	28	Daugava	Latvia	5	550	555	11.4
23	28	Venta	Latvia	15	19	34	0.7
	27	Emån	Sweden	4		4	0.1
24	25	Mörrumsån	Sweden	30		30	0.6
	26	Neumunas	Lithuania	5		5	0.1
Total				288	4583	4870.3	100.0

Note: Estonian, Finnish, and Swedish wild productions are from the ICES report (Anonymous 1996). Russian data are according to Anatsky and Dirin (1997), Latvian production data according to Oleg Vasin, and Lithuanian data according to Antanas Kontautas. Hatchery production data of Finland are from Finnish Game and Fisheries Research Institute (unpublished data), of Sweden are from Salmon Research Institute, and of Estonia according to Tiit Paaver.

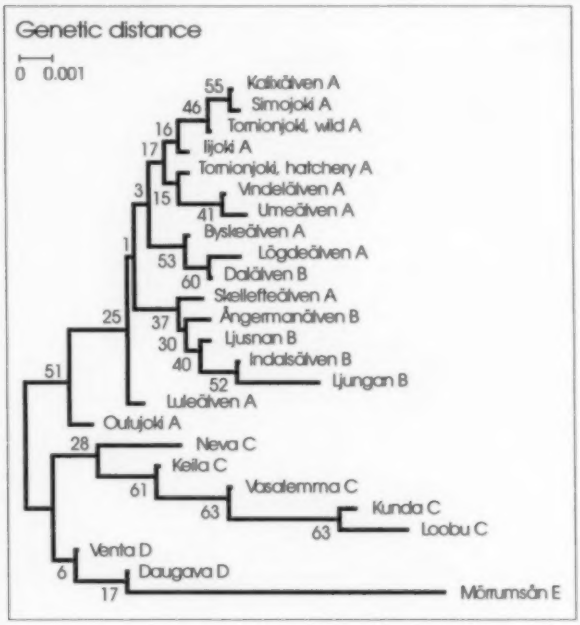
linear negative regression would be expected, and some kind of balance between gene flow and genetic drift would exist. These analyses were based on data from 22 populations. Data from those of Kalixälven, Ljungan, and Lögde were excluded because the individual genotype data needed for GENEPOP analyses were not available for these stocks.

Unbiased expected mean heterozygosities (for all 18 loci) and their standard errors were estimated according to Nei (1978). To examine the source of the variation in diversity estimates, standard errors for average diversities were calculated separately for the total variation and for the intralocus variation component (Nei 1987). In general, the mean heterozygosities of populations were compared by the *t* test for paired observations because allele frequencies and heterozygosities of conspecific populations are historically correlated (Nei 1987). For some samples, the test for individual loci according to Nei (1987) was also conducted. In the case of the Tornionjoki stock, in which only one generation separates the wild and hatchery populations compared, the historical evolutionary differences can be excluded, and the test could therefore be restricted to the particular set of loci studied and the normal deviate test (Nei 1987) could be used.

The distribution of the genetic diversity of salmon stocks was examined with the hierarchical diversity analysis of Nei (1977). To attain consistency with the assumption of equal weights for populations, the average over groups was determined with weights proportional to the number of stocks in the group (Chakraborty and Leimar 1987). The stocks of the rivers where salmon have access to spawning grounds and can reproduce naturally were classified as wild stocks, even though supportive fish releases have been carried out in some of the rivers (Table 1). The material for supportive releases was derived from either local returning spawners or hatchery broodstocks created from local spawners.

© 1999 NRC Canada

Fig. 2. Genetic distances of Baltic Atlantic salmon stocks. The letters A–E correspond to the sea areas from which the stocks originate (see Fig. 1).



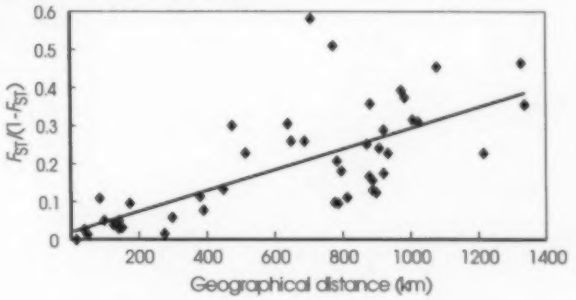
Results

Geographical distribution of genetic differentiation

The genetic distances between populations from geographically different areas were substantial, and a dichotomy was seen between stocks from the east and those from the west (Fig. 2). This dichotomy was largely due to variation in the *IDDH-2** locus. The frequency of the allele *IDDH-2*–72* was markedly higher (0.70–0.83) in the three southeastern areas (Gulf of Finland, Eastern Main Basin, Southern Main Basin) than in the northwestern areas (0.18–0.51) (Bothnian Sea, Bothnian Bay) (Table 3). At other loci, Mörrumsån (Southern Main Basin) had an exceptionally high frequency of allele **116* in *IDHP-3** (0.49). In Estonian stocks, the frequencies of *mMEP-2*125* (0.28–0.70) were higher than in Baltic Sea stocks in general (0.01–0.18) (Table 3). Thus, of the three alternative hypotheses of colonisation, genetic distance between stocks was the most consistent with the hypothesis of the colonisation of the Baltic Sea region by two different phylogeographic lineages. The eastern lineage covered stocks of the Main Basin and Gulf of Finland and the western lineage those of the Gulf of Bothnia.

The dendrogram also matched the present geographical form of the Baltic Sea. The populations were clustered into three branches corresponding to the three main parts of the sea from which they originated: Gulf of Bothnia (Bothnian Bay (A), Bothnian Sea (B)), Gulf of Finland (C), and Main Basin (Eastern Main Basin (D), Southern Main Basin (E)) (Fig. 2). Within the Gulf of Bothnia group, the stocks from its two parts Bothnian Bay and Bothnian Sea grouped partly together, yet with some differentiation between them. Within the Main Basin, the Mörrumsån stock clearly deviated from the Latvian salmon stocks of the Eastern Main Basin.

Fig. 3. Relationship of pairwise genetic distances and geographical distances of Baltic Atlantic salmon stocks to the one-dimensional dispersal pattern.



Allele frequency differences were highly significant for all loci throughout the Baltic Sea. Population differentiation was also highly significant ($p < 0.001$) within the Gulf of Bothnia, Bothnian Sea, and Gulf of Finland and significant ($p < 0.05$) within the Eastern Main Basin. The great majority of all pairwise tests for contingency between stocks were highly significant. In only nine out of 625 tested pairs could a statistically significant difference not be observed, at least at the 5% level.

Most of the populations studied were in Hardy–Weinberg equilibrium. After Bonferroni correction for several loci per population, statistically significant deviations were seen in four (all hatchery samples) out of 152 of the tests: an excess of heterozygotes in the Tornionjoki 1993 sample (*IDDH-2**, $p = 0.024$) and a deficiency of heterozygotes in Neva 1990 (*IDDH-1**, $p < 0.001$), Skellefteälven (*IDDH-2**, $p = 0.025$), and Ångermanälven (*IDDH-2**, $p = 0.025$). At stock level, when the probabilities of different loci were joint with Fisher's method, the only statistically significant deviations were in samples from hatchery stocks of Skellefteälven ($p = 0.03$) and Neva 1990 ($p < 0.001$).

The geographical and genetic distances of Baltic salmon stock pairs fitted the isolation-by-distance model. According to the Mantel test, the correspondence was highly significant ($p < 0.001$) for all stocks studied and for wild stocks; there was, however, no correspondence ($p = 0.14$) if only hatchery stocks were considered. The observed genetic distances also fitted the isolation-by-distance pattern when the two lineages were treated separately. For the eastern lineage the correlation was highly significant ($p < 0.001$) and for the western lineage was significant ($p = 0.027$). The data on wild stocks had a better fit with the regression model in which $F_{ST}/(1 - F_{ST})$ values were plotted directly against geographical distance (regression coefficient 0.70, Fig. 3) rather than on its natural logarithm (regression coefficient 0.66), indicating that the dispersal of salmon stocks bears a closer resemblance to the one- than to the two-dimensional pattern (Slatkin 1993).

The apparent pattern of isolation-by-distance among Baltic salmon populations could also be seen when the F_{ST} values were transformed into estimates of gene flow as number of migrants by plotting the log(migrants) on log(geographical distance) from all population pairs of wild and hatchery populations (Fig. 4*a*). The linear regression line was then

© 1999 NRC Canada

Table 3. Allele frequencies in variable loci in Baltic salmon stock samples.

	*sAAT4**				*IDDH-1**			*IDDH-2**		
Stock	*n*	*100*	*25*	*50*	*n*	*100*	*28*	*n*	*100*	*72*
31. Bothnian Bay										
1a. Tornionjoki W	641	0.732	0.265	0.003	645	0.998	0.002	643	0.658	0.342
1b. Tornionjoki H	579	0.904	0.096	0	556	1	0	512	0.673	0.327
2. Simojoki	653	0.643	0.353	0.004	700	1	0	643	0.632	0.368
3. Iijoki	284	0.794	0.206	0	374	0.995	0.005	351	0.620	0.380
4. Oulujoki	230	0.770	0.215	0.015	239	0.969	0.031	230	0.596	0.404
5. Kalix	719	0.677	0.323	0	764	1	0	764	0.673	0.327
6. Luleälven	60	0.825	0.133	0.042	60	0.975	0.025	60	0.650	0.350
7. Byskeälven	29	0.931	0.069	0	26	0.981	0.019	30	0.583	0.417
8 Skellefteälven	84	0.893	0.101	0.006	84	0.946	0.054	84	0.494	0.506
9 Vindelälven	100	0.850	0.150	0	100	1	0	100	0.760	0.240
10. Umeälven	98	0.893	0.107	0	89	0.995	0.005	97	0.820	0.180
11. Lögdeälven[a]	69	0.986	0.014	0	69	0.987	0.013	69	0.552	0.448
30. Bothnian Sea										
12. Ångermanälven	100	0.930	0.070	0	100	0.940	0.060	100	0.510	0.490
13. Indalsälven	89	0.949	0.051	0	88	0.892	0.108	88	0.733	0.267
14. Ljungan	51	0.804	0.196	0	52	0.750	0.250	52	0.788	0.212
15. Ljusnan	50	0.840	0.160	0	50	0.900	0.100	50	0.560	0.440
16. Dalälven	200	0.960	0.040	0	200	0.987	0.013	196	0.561	0.439
32. Gulf of Finland										
17. Neva	170	0.691	0.229	0.080	320	0.925	0.075	320	0.166	0.834
18. Kunda	40	0.587	0.413	0	25	0.980	0.020	42	0.250	0.750
19. Loobu	35	0.772	0.214	0.014	15	1	0	33	0.303	0.697
20. Keila	40	0.625	0.325	0.050	22	0.977	0.023	40	0.300	0.700
21. Vasalemma	30	0.750	0.233	0.017	7	1	0	30	0.283	0.717
28. Eastern Main Basin										
22. Daugava	125	0.804	0.196	0	88	1	0	125	0.232	0.768
23. Venta	107	0.678	0.322	0	94	1	0	107	0.299	0.701
25. Southern Main Basin										
24. Mörrumsån	67	0.746	0.254	0	57	0.991	0.009	67	0.231	0.769
Total	4650				4824			4833		

[a]Data are from Ståhl (1983).

log(migrants) = 2.96–0.68 log(distance (km)). The regression explained 69% of the variation in the data. When only the 11 wild stocks were included, the regression line was very similar, log(migrants) = 2.82–0.82 log(distance), whereas the coefficient of determination was as high as 82% of the observed variation (Fig. 4*b*). For the hatchery stock group, the isolation-by-distance model did not fit the data. Hatchery production also caused increased scattering of the data points, as seen in the figure for joint hatchery and wild production (Fig. 4*a*).

The population distances studied covered the range from over 100 migrants to zero. Most of the population pairs were separated by distances of between 100 and 1000 km. At this range, the estimated number of migrants decreased from about 10 to near zero (Fig. 4*a*). The migration rate between several population pairs from distances of over 600 km (2.6 in log scale) was already very low, near the one individual per generation level (0 in log scale) (Fig. 4).

Mean diversities of populations and groups

In the diversity analyses the hierarchical levels used were total, lineage, area, and stock. Within the whole Baltic Sea, the proportion of the between-stock diversity component was a substantial 15% (G_{ST} = 0.147) (Table 4). As much as half of this was a consequence of differentiation between the two proposed phylogeographic lineages (G_{LT} = 7.5%), but differences between sea areas within lineages were also apparent (G_{AL} = 2.9%). Together, these upper hierarchical levels constituted two thirds of the between-stock component.

The total diversity was almost 30% higher for the wild group (H_T = 0.076) than for the hatchery group (H_T = 0.054) (Table 4). This was explained partly by the dissimilar distributions of wild and hatchery stocks among sea areas and partly by the historically different levels of diversity within these areas. This could also be seen in the substantially higher G_{ST} estimate (G_{ST} = 0.161) for diversity between wild stocks than between hatchery stocks (G_{ST} = 0.063). Within Bothnian Bay alone, where historically more similar genotype structures could be expected, the level was the same. For this area, H_T for the six wild stocks was 0.057 and for the six hatchery stocks was 0.055.

Diversities within populations, measured as mean expected heterozygosities for 18 loci, varied from 3 to 8.2% (Table 5). The average mean heterozygosity was somewhat higher for the wild stocks (6.3%) than for the hatchery stocks (5.1%), but the difference was not significant (t = 1.536, df = 6, one-tailed $p < 0.10$). When the mean heterozygosities of popula-

© 1999 NRC Canada

*IDHP-3**			*sMDH-3,4**				*mMEP-2**			*PGM-1**		
n	*100*	*116*	*n*	*100*	*80*	*115*	*n*	*100*	*125*	*n*	*100*	*75*
645	0.997	0.003	645	0.992	0.003	0.005	645	0.898	0.102	646	0.980	0.020
502	0.996	0.004	555	0.988	0.009	0.003	571	0.901	0.099	555	0.990	0.010
706	0.999	0.001	749	0.998	0.002	0	747	0.886	0.114	747	0.991	0.009
365	1	0	393	0.982	0.018	0	382	0.853	0.147	393	1	0
208	0.887	0.113	240	0.998	0.002	0	238	0.847	0.153	240	0.998	0.002
773	1	0	773	0.995	0.005	0	745	0.917	0.083	756	0.988	0.012
60	0.992	0.008	59	0.992	0	0.008	59	0.907	0.093	60	1	0
30	1	0	30	1	0	0	30	0.817	0.183	30	0.983	0.017
84	1	0	84	0.994	0.006	0	84	0.988	0.012	84	1	0
100	1	0	100	1	0	0	100	0.950	0.050	100	1	0
98	1	0	98	1	0	0	98	0.980	0.020	98	1	0
69	1	0	69	1	0	0	69	0.819	0.181	69	1	0
100	0.955	0.045	100	1	0	0	100	0.980	0.020	100	1	0
89	0.994	0.006	89	1	0	0	89	0.972	0.028	89	1	0
52	1	0	52	1	0	0	52	0.962	0.038	52	1	0
50	0.980	0.020	50	1	0	0	50	0.980	0.020	50	1	0
200	1	0	200	1	0	0	200	0.880	0.120	200	1	0
316	0.979	0.021	320	0.997	0.003	0	319	0.878	0.122	320	1	0
40	1	0	59	1	0	0	58	0.431	0.569	59	1	0
36	1	0	50	1	0	0	50	0.300	0.700	50	1	0
39	1	0	63	1	0	0	63	0.714	0.286	63	1	0
30	1	0	43	1	0	0	43	0.616	0.384	43	1	0
125	0.964	0.036	125	1	0	0	125	0.904	0.096	125	0.996	0.004
103	0.981	0.019	107	1	0	0	107	0.902	0.098	107	1	0
65	0.515	0.485	67	1	0	0	67	0.881	0.119	67	1	0
4885			5120				5091			5103		

tion pairs were compared at the total genome level by the pairwise *t* test, which includes the between-loci variation component, no statistically significant differences ($p > 95\%$) were found between any of the population pairs, not even between Mörrumsån (8.2%) and Umeälven (3.0%) ($t = 2.17$, for five variable loci, df = 4, two-tailed $p < 0.10$). Differences were, however, statistically significant between these stocks at the locus level in four out of five individual loci at $p < 0.001$: *IDDH-1**, *IDDH-2**, *IDHP-3**, and *mMEP-2**. Similarly, several other stock pairs displayed significant differences at the locus level but not at the total genome level, although allele frequency differences between population pairs were statistically significant in most cases. The variation in standard errors was mainly due to the variation between loci (Table 5).

The mean heterozygosity of the Tornionjoki hatchery strain was lower (0.47) than, but not significantly different from, that of the wild strain (0.61) ($t = 1.640$, df = 6, one-tailed $p < 0.10$) for the total genome level. The heterozygosity of three individual loci was, however, significantly lower in the Tornionjoki hatchery strain than in the wild stock sample (*sAAT-4**, $p < 0.001$; *PGM-1**, $p < 0.05$; *IDDH-1**, $p < 0.001$). When only the set of the variable loci studied was included, and only intralocus variances were used, the normal deviate test showed that the Tornionjoki hatchery strain had a lower mean heterozygosity at $p < 0.001$ in these loci. In addition, allele frequency differences between the two Tornionjoki strains were highly significant in four out of seven loci (*sAAT-4**, $p = 0.000$; *IDDH-2**, $p = 0.000$; *sMDH-3,4**, $p = 0.0017$; *PGM-1**, $p = 0.0012$).

When the genetic variability was measured as the number of different alleles, a somewhat different picture emerged. For example, small Estonian stocks had quite high levels of heterozygosity but a low number of alleles. Hatchery stocks had, on average, even slightly higher values than wild stocks (Table 5). The Tornionjoki wild stock, however, was the only one having all observed potential 16 alleles (average 2.3) in the seven polymorphic loci. The highest number of alleles was in stocks of the Bothnian Bay area.

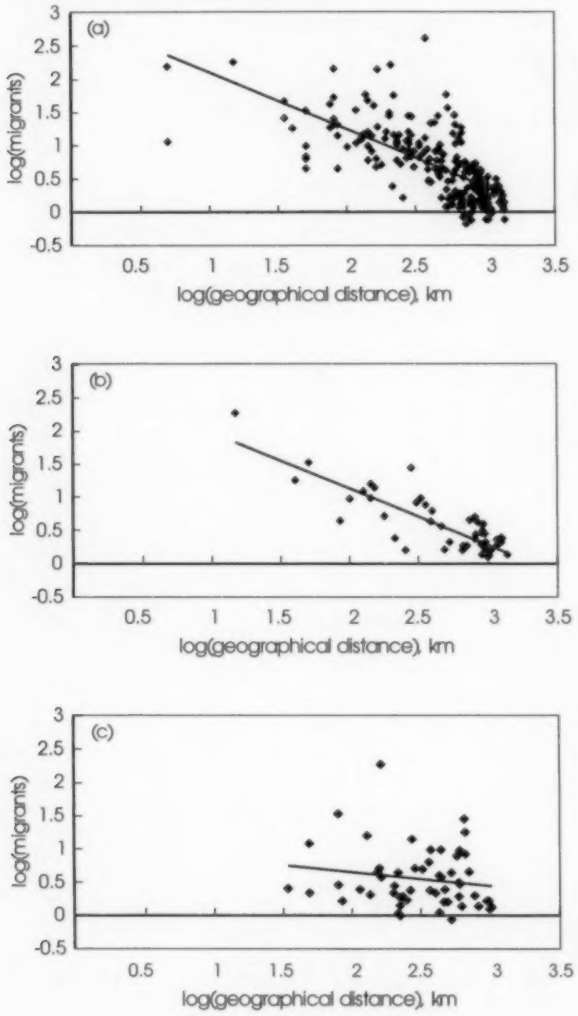
Discussion

Pattern of genetic differentiation of populations

The genetic distances of stocks within the Baltic Sea showed a dichotomy between northwestern and southeastern stocks (Fig. 2) that was consistent with the hypothesis of two distinct postglacial colonisation lineages. When the allele frequencies of the potential source populations outside the Baltic Sea were compared with those of the corresponding

© 1999 NRC Canada

Fig. 4. Relationship of gene flow (log (migrants)) to geographical distance (log (geographical distance (km))) for (*a*) wild and hatchery Atlantic salmon stocks, (*b*) wild stocks alone, and (*c*) hatchery stocks alone.



lineages within the Baltic Sea, the results gave further support to this hypothesis of colonisation from both east and west. In our data, within the Baltic Sea, the frequency of the allele *IDDH-2*–72* was clearly higher in the eastern lineage (0.70–0.83) than in the western lineage (0.18–0.50). The frequency of this same allele was, however, still higher in the eastern landlocked populations: 1.00 in Lake Onega (Koljonen 1989) and 0.92 in Lake Ladoga (Kazakov and Titov 1993) (Fig. 1). The similarity between these Russian landlocked populations and the southeastern lineage within the Baltic Sea was evident.

An Atlantic salmon stock from the Atlantic, Numedalslågen, had a frequency of 0.36 of allele *–72 (Bourke et al. 1997), which is identical to the mean of our data from the stocks in the Gulf of Bothnia area. In their dendrogram, this Norwegian salmon stock also groups together with the salmon stocks from the Gulf of Bothnia (Tornionjoki, Simojoki, Dalälven, Luleälven; Bourke et al. 1997). In the nearest Atlantic populations, i.e., those of Ireland and Scotland, the frequency of this allele was very similar to that of the Gulf of Bothnia (0.38; Bourke et al. 1997). As a result, the allele frequencies of the stocks from the southeastern part of the Baltic Sea (Russia, Estonia, Latvia, southern Sweden) were closer to those of the eastern, landlocked stocks (Lakes Onega and Ladoga) than to those of the northwestern Baltic salmon stocks around the Gulf of Bothnia (northern Finland, northern Sweden), which in turn bore a closer resemblance to the stocks from rivers draining into the North Sea and eastern Atlantic (Fig. 5). The hypothesis was also supported by the finding that genetic distances for variable allozyme loci among stocks from the coast of continental Europe and the northern Baltic were under 0.03 (Bourke et al. 1997) but that the average distance within the Baltic Sea between the northwestern group and Neva stocks was 0.045.

That the present dispersal areas of the western and eastern lineages correspond to the extent of the Baltic Sea stages in which dispersal could have been possible during glaciation provides further support for the hypothesis that dispersal occurred in two directions and at different times. The range of the stocks of the eastern lineage is compatible with the extent of the first stage of the Baltic Sea, the Baltic Ice Lake, which formed at about 12 200 – 12 000 years BP, when the ice-dammed lakes in the eastern Gulf of Finland were joined with the rest of the Baltic basin (Donner 1995). Until the end of the Baltic Ice Lake stage, the Baltic Sea was a lake and the ice sheet had not yet retreated from Finland and northern Sweden (Fig. 6*a*). Even on the south coast of Sweden, the Rivers Emån (0.65; Ståhl 1987; Fig. 1) and Mörrumsån have high frequencies of the eastern allele (*–72) and thus seem to come within the range of influence of the eastern lineage.

Lake Ladoga was at that time part of the Baltic Ice Lake, which explains the similarity between salmon from Lake Ladoga and the stocks of the eastern lineage. Lake Onega was isolated from the preceding River Onega Ice Lake before the Baltic Ice Lake stage (12 500 – 11 500 BP) (Saarnisto et al. 1995) and drained into Lake Ladoga. The existence of landlocked salmon in Lake Onega indicates that they had survived in glacial lakes. The exceptional allele frequency (0.49) of allele *IDHP-3*116* in the Mörrumsån stock could not be explained by gene flow from the North Sea, as high frequencies were not observed there, the range being from 0.00 to 0.20 (Bourke et al. 1997). This exception is therefore attributed to genetic drift in an isolated population. The eastern lineage of the Baltic Sea stocks together with the present landlocked stocks from Lakes Ladoga and Onega is here called the Ice Lake lineage.

The initial provenance of the eastern lineage was most likely glacial ice lakes in present-day Russia. Segerstråle (1976) proposed that the Ice Lake of the River Onega basin (Fig. 6) and glacial lakes along the edge of the continental ice sheet served as refugia for glacial relicts of the Baltic Sea area, e.g., the fourhorn sculpin (*Myoxocephalus quadricornis*), a fish that had colonised Finland via this eastern route. The outer margin of the ice sheet was less than 80 km southeast of Lake Onega (Saarnisto et al. 1995). From this refugium area, salmon were able to colonise both Lakes

© 1999 NRC Canada

Table 4. Hierarchical diversity analysis of Baltic Atlantic salmon stocks.

Stock group	K	H_T	H_L	H_A	H_S	G_{ST}	G_{SL}	G_{SA}	G_{AL}	G_{AT}	G_{LT}
Whole Baltic Sea	25	0.067	0.062	0.060	0.058	0.147	0.075	0.042	0.029	0.105	0.075
Lineage											
Atlantic	17		0.055	0.055	0.053		0.047	0.040	0.007		
Ice Lake	8		0.077	0.072	0.063		0.108	0.040	0.069		
Sea area											
31. Bothnian Bay	12			0.056	0.054			0.039			
30. Bothnian Sea	5			0.053	0.051			0.044			
32. Gulf of Finland	5			0.076	0.072			0.064			
28. Eastern Main Basin	2			0.056	0.056			0.006			
25. Mörrumsån	1			0.081	0.081						
Wild stocks	14	0.076			0.063	0.161					
Hatchery stocks	11	0.054			0.051	0.063					

Note: Distribution of total diversity was also examined separately for wild and hatchery stock groups. K, number of stocks within each grouping (relative weight of group in analysis); H_T, total diversity; H_L, diversity for lineages; H_A, diversity for sea areas; H_S,diversity for samples. Estimates of G are the proportions of between-stock diversity components between corresponding hierarchical levels: G_{ST}, in the whole data; G_{SL}, between stocks within lineages; G_{SA}, between stocks within areas; G_{AL}, between sea areas within lineages; G_{AT}, between sea areas; G_{LT}, between lineages.

Onega and Ladoga and also the southeastern Baltic Sea rivers. The homing behaviour of salmon has played a role in maintaining the patterns of this first colonisation.

Salmon stocks of the Gulf of Bothnia from north of central Sweden formed the western lineage, the range of which corresponded to the geographical region that was liberated from ice after the Baltic Ice Lake stage, i.e., at the Yoldia Sea stage. This stage began when a wide sound opened across central Sweden over the large basins of Lakes Vänern and Vättern, via the Närke strait, and the first great ingress of seawater turned the water of the Baltic Sea brackish (Fig. 6*b*). This period lasted from about 10 300 to 9000 BP (Donner 1995), i.e., until the sea connection was closed and a second, shorter lake period, the Ancylus Lake, began. During the Yoldia Sea stage the northern Baltic Sea coasts could have been colonised by salmon from the North Sea. At that time, Finland and most of the present Swedish coast were already free of ice, and spawning habitats were available in rivers (Fig. 6*b*). The western lineage is suggested to have colonised along this route and is called the Atlantic lineage. At the Yoldia Sea stage, other marine fauna, also, such as molluscs and crustaceans, colonised the Baltic Sea area (Donner 1995). This route was probably also used by the ringed seal (*Phoca hispida*) (the subspecies in the Baltic Sea is *Phoca hispida botnica*) to invade the Baltic Sea (Forstén and Alhonen 1975). It is possible that some salmon of the Atlantic lineage entered the Baltic Sea later, during the Littorina Sea stage, via the sounds between Sweden and Denmark (about 9000 BP; Donner 1995), and afterwards.

The Finnish salmon of Lake Saimaa are closer to the stocks of the Atlantic Lineage (the frequency of the *–72 allele was 0.27; M.-L. Koljonen, unpublished) and, moreover, the western colonisation route was also possible for this population. The Lake Saimaa area was covered by ice at the time of the Baltic Ice Lake (Fig. 6*a*) and its first outlet, at about 8000 years BP, was northwestwards into the Gulf of Bothnia. The secondary outlet, into Lake Ladoga, did not form until about 5000 years BP (Saarnisto 1970). Thus, this landlocked population derived originally from an ancient salmon population in the Gulf of Bothnia area. Lake Saimaa also hosts a landlocked ringed seal (*Phoca hispida saimensii*) population, which is assumed to be of western origin (Forstén and Alhonen 1975).

The hypothesis of two phylogeographic colonisation lineages was supported by three factors: (*i*) possible donor populations and colonisation routes existed for both lineages, (*ii*) the lineages in the Baltic Sea were genetically more similar to their postulated donor stocks outside the present Baltic Sea than to each other, and the genetic distance between these lineages was considerable, and (*iii*) the ranges of the suggested lineages within the Baltic Sea were compatible with the glaciation stages at which colonisation is expected to have occurred.

In light of the above colonisation history, the oldest and more rare genetic material for this area is currently to be found in the present salmon stocks of Estonia, Latvia, Lithuania, Russia, and southern Sweden in the Ice Lake lineage. The freshwater period was markedly longer, over 60 000 years, in the evolution of this lineage than in that of the Atlantic lineage of northern Finland and northern Sweden. The risk of the Ice Lake lineage becoming extinct is greater than is that of the Atlantic lineage within the Baltic Sea, as indicated by current wild smolt production levels (Table 2). The genetic adaptation of all Baltic salmon to a freshwater (brackish) environment is demonstrated by their genetic resistance to *Gyrodactylus salaris* (Bakke et al. 1990), a fatal freshwater ectoparasite that is responsible for the death of several Atlantic salmon stocks in Norway. This parasite was introduced into Norway accidentally in the course of a stock transfer from the Baltic region. A vital proportion of the genetic diversity (with likely adaptive differences in, say, migration behaviour) within the Baltic salmon lies between these two lineages. They have evolved relatively independently until now and their existence should be taken into account in efforts to conserve the genetic diversity of the Baltic salmon.

The genetic differentiation between sea areas, within the phylogeographic lineages, is due partly to the longer geographical distances and partly to the more recent differences in the form and environment of the Baltic Sea; thus, it represents a different aspect of genetic differentiation from that caused by ancient glacial isolation and old colonisation routes.

© 1999 NRC Canada

Table 5. Mean number of alleles for seven loci and standard errors and mean heterozygosities for 18 loci and their standard errors (SE total, including variation both between and within loci; SE sample, excluding variation between loci).

Stock	Origin	Country	Alleles for seven loci	SE	Heterozygosities for 18 loci	SE total	SE sample
31. Bothnian Bay							
1a. Tornionjoki, W	Wild	Finland, Sweden	2.3	0.18	0.061	0.033	0.0020
1b. Tornionjoki, H	Hatchery	Finland	2.0	0.22	0.047	0.027	0.0022
2. Simojoki	Wild	Finland	2.0	0.22	0.064	0.036	0.0016
3. Iijoki	Hatchery	Finland	1.7	0.18	0.061	0.033	0.0027
4. Oulujoki	Hatchery	Finland	2.1	0.14	0.076	0.035	0.0039
5. Kalixälven	Wild	Sweden	1.7	0.18	0.059	0.034	0.0016
6. Luleälven	Hatchery	Sweden	2.0	0.22	0.056	0.030	0.0071
7. Byskeälven	Wild	Sweden	1.7	0.18	0.056	0.031	0.0090
8. Skellefteälven	Hatchery	Sweden	1.9	0.26	0.046	0.029	0.0048
9. Vindelälven	Wild	Sweden	1.4	0.20	0.040	0.024	0.0049
10. Umeälven	Hatchery	Sweden	1.6	0.20	0.030	0.019	0.0048
11. Lögdeälven	Wild	Sweden	1.6	0.20	0.047	0.031	0.0045
Mean			1.8		0.054		
30. Bothnian Sea							
12. Ångermanälven	Hatchery	Sweden	1.7	0.18	0.048	0.029	0.0048
13. Indalsälven	Hatchery	Sweden	1.7	0.18	0.042	0.024	0.0055
14. Ljungan	Wild	Sweden	1.6	0.18	0.062	0.031	0.0077
15. Ljusnan	Hatchery	Sweden	1.7	0.20	0.057	0.031	0.0072
16. Dalälven	Hatchery	Sweden	1.6	0.20	0.045	0.029	0.0030
Mean			1.7		0.051		
32. Gulf of Finland							
17. Neva	Wild	Russia	2.0	0.22	0.064	0.031	0.0039
18. Kunda	Wild	Estonia	1.6	0.20	0.078	0.041	0.0059
19. Loobu	Wild	Estonia	1.6	0.30	0.068	0.037	0.0073
20. Keila	Wild	Estonia	1.7	0.29	0.077	0.041	0.0071
21. Vasalemma	Wild	Estonia	1.6	0.30	0.071	0.039	0.0074
Mean			1.7		0.072		
28. Eastern Main Basin							
22. Daugava	Hatchery	Latvia	1.7	0.18	0.051	0.027	0.0050
23. Venta	Wild	Latvia	1.6	0.20	0.060	0.033	0.0045
Mean			1.7		0.056		
25. Southern Main Basin							
24. Mörrumsån	Wild	Sweden	1.7	0.18	0.082	0.038	0.0064
Total mean			1.75		0.058		
Mean wild			1.7		0.063		
Mean hatchery			1.8		0.051		

It nevertheless made a clear contribution to the total diversity (one fifth of the between-stock component, Table 4).

The differences observed between populations within each sea area, the lowest level in the hierarchy of the genetic structure, were systematically statistically significant, yet not generally very large. The marked deviations between the Oulujoki and Luleälven stocks and the other Gulf of Bothnia stocks are due to mixing of stocks in the rearing process. Thus, this human interference was seen as deviations from the expected differentiation pattern. In Latvia, salmon from the River Venta very likely resemble those of the River Daugava more than historically because both eggs and juveniles have been transferred from the Daugava to the Venta over a long period.

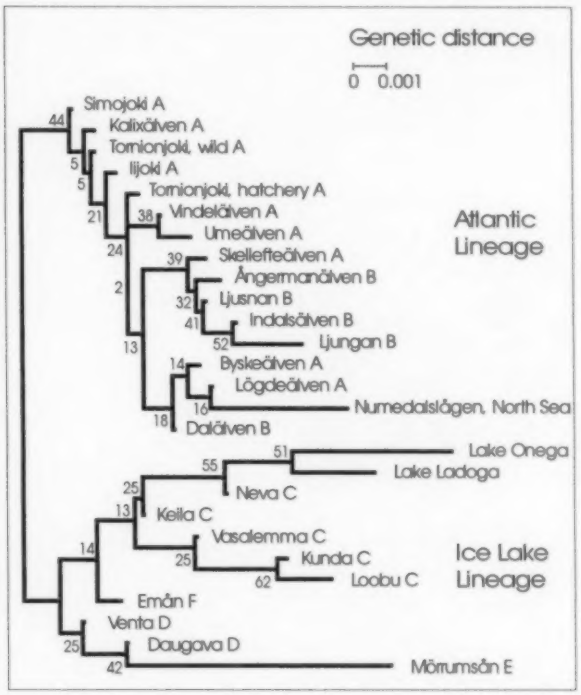
Isolation pattern and rate of gene flow

The pattern of differentiation of Baltic Sea salmon populations fits the isolation-by-distance model (Wright 1943; Kimura and Weiss 1964). Migrant dispersal was restricted to short distances and was thus most common between geographically adjacent stocks. So, although all stocks feed in the Baltic Main Basin, spawners tend mainly to stray to the neighbouring river along the shore.

The observed differentiation pattern fitted the one-dimensional migration pattern better than the two-dimensional plane pattern. It can be described by the theoretical one-dimensional "stepping stone" model (Kimura 1953), according to which populations are located as "steps" on a line, and in each generation, an individual can migrate a restricted number of "steps" in either direction along the line (Kimura and Weiss 1964). Considerable local differentiation will be more readily expected in the one-dimensional than in the two-dimensional migration pattern, and in this model the correlation between geographical distance and genetic dis-

© 1999 NRC Canada

Fig. 5. Genetic distances between Atlantic salmon stocks when populations outside the present Baltic Sea were added. The letters A–E refer to the sea areas from which the stocks originate.



tance also holds for longer geographical distances than in the two-dimensional model (Kimura and Weiss 1964). Thus, the location of river mouths along the shore, in a line, and over long distances offers relatively good prospects for salmon stocks to develop local adaptations. The occurrence of this pattern indicates the relatively neutral nature of allozyme variation.

The rate of gene flow was very low for distances of over 600 km; since geographical distances between the proposed phylogenetic lineages usually exceeded 550 km, there was very little gene flow between populations from different lineages. The geographical location of stocks corresponds roughly to that of two lines apart from each other. For shorter distances, gene flow had clearly occurred, and although our estimates are rough, they indicate that the role of gene flow has probably been underestimated in maintaining diversity among populations.

The occurrence of the isolation-by-distance pattern has only recently been tested on Atlantic salmon, previous authors having stressed the completely independent evolution of each river stock (e.g., Fontaine et al. 1997). Isolation by distance has been found to occur in salmon populations over long distances in data from west Atlantic populations in eastern Canada (McConnell et al. 1997) and from east Atlantic populations from Norway to Spain (Blanco et al. 1992; Bourke et al. 1997). In addition, isolation was observed over shorter geographical distances in Danish Atlantic salmon stocks from 1913, before human interference changed the population structure (Nielsen et al. 1999).

Before human impact, the population structure of Baltic Sea salmon most likely corresponded better to the isolation-by-distance pattern than it does today. A balance probably prevailed right up until the 1930s, when damming of the big rivers resulted in the beginning of fish rearing, which tends to mix populations. Thus, despite the many human-induced changes in the genetic structure of salmon populations, isolation by distance can still be recognised in the population differentiation pattern.

Amount of genetic diversity

The total diversity (H_T) of natural stocks in the recent Baltic Sea is still about 30% higher (7.6%) than that of all hatchery stocks together (5.7%). Despite their weak and threatened status, recent natural stocks make a very marked contribution to the genetic biodiversity of Baltic salmon, especially those of the Ice Lake lineage. Recent hatchery stocks cannot completely replace the genetic resources of these stocks. When the hatchery and wild stock groups of the Bothnian Sea alone were compared, the diversity levels were about the same, showing that by using captive breeding in hatcheries, it is possible to maintain genetic variability (number of alleles) at least at a higher level than in the smallest wild stocks.

Population sizes tend to be smaller in ordinary hatchery stocks than in a medium-sized natural salmon stock, which causes loss of genetic variation. An effective size of 1000 individuals is large for a hatchery stock, but big natural stocks can have as many as tens of thousands of spawners. The smallest Estonian wild stocks, however, have fewer than 100 spawners a year. In the Tornionjoki stock case, two out of 16 alleles (12.5%) were lost in broodstock sampling.

Rare alleles have, however, very likely been lost in the small natural stocks as well, e.g., those of Byskeälven and Lögdeälven. The small Estonian stocks (Kunda, Loobu, Keila, Vasalemma) also seemed to have fewer rare alleles than the Neva stock of the same area (Table 3). The loss of alleles could be expected to be a direct consequence of small population size and to occur in both natural and hatchery populations when they are small and isolated. The small population sizes and isolated status of wild stocks are often a result of human activity, which is thus also responsible for these losses. In the Gulf of Bothnia, only the population sizes of the Tornionjoki stock (75 000 smolts) have succeeded in maintaining all potential (found) alleles. Management strategies should therefore take account of the risk of losing diversity arising from both cases.

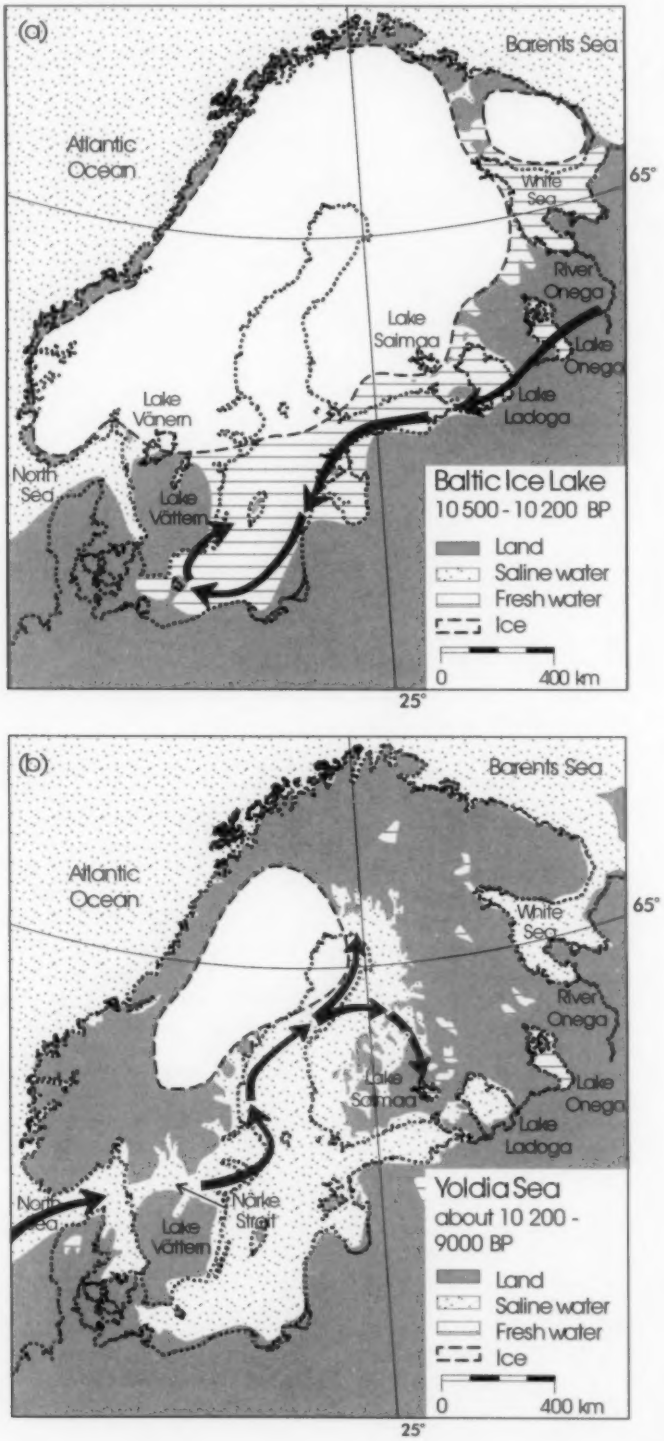
Because of the limited number of variable loci available, allozyme studies in general enable only severe bottlenecks to be established as statistically significant changes in mean heterozygosities. To increase the power of the tests for mean heterozygosities, McCommas and Bryant (1990) estimated that for an 80% probability of detecting a bottleneck at 5% significance, 15 loci were needed for a bottleneck size of four reproductive pairs and as many as 46 loci for 16 pairs when 30 individuals per population were analysed. The single-locus tests revealed, however, that changes in particular loci had clearly taken place.

Management implications

The maintenance of genetic diversity is internationally accepted as a common long-term goal for the management of

© 1999 NRC Canada

Fig. 6. (*a*) Proposed route by which the Ice Lake lineage of Atlantic salmon colonised Baltic Sea rivers at the Baltic Ice Lake stage. The final phase of the Baltic Sea Lake stage before the marine connection to the North Sea. Modified from a map by Eronen and Haila (Alalammi 1992). (*b*) The proposed route by which the Atlantic lineage of Atlantic salmon colonised Baltic Sea rivers at the Yoldia Sea stage. Modified from maps by Eronen and Haila (Alalammi 1992).



© 1999 NRC Canada

all living natural resources (United Nations 1992). It has also been stated often and unequivocally that the conservation of genetic diversity requires conservation of both levels of diversity, that between and that within stocks (e.g., Waples 1991; Ryman et al. 1995). The conservation strategies of these two components of diversity are, in practice, often opposed to each other, as the first seeks to avoid gene flow and the second loss of variation due to small population sizes, possibly with aid of organized gene flow.

When considering management principles for Baltic salmon, in the longest term, we should ensure that genetic material is preserved throughout the Baltic Sea range with a diversity and viability sufficient to permit the continuous evolution of self-sustaining populations of this isolated, brackish water adapted form of salmon. With the harnessing of reproduction rivers, many salmon stocks and their habitats have been irreversibly lost, and the recent Baltic salmon gene pool is only a fraction of what it was before human impact (only about 25 out of 90 stocks remain). There can be no return to the original state of Baltic salmon stocks; we can only safeguard their continuous evolution from the present state, in an environment with limited potential for natural reproduction. It is therefore crucial that we maintain what remains of the total gene pool after glaciations and human impact to as large an extent as possible. The long-term conservation goal thus gives priority to future evolutionary potential over attained adaptation, should these two come into conflict.

The scope for enhancement projects is limited by the range of potential reproduction areas, making it all the more important to locate them in areas where natural reproduction can still take place at a relatively high level and with considerable population sizes. It would be important to ensure the effective use of (at least) these areas for natural salmon reproduction.

The genetic resources of Baltic salmon derive from both wild and hatchery stock groups, one group complementing the other. The long-term conservation strategy should therefore seek to achieve a match between recent genetic material and potential reproduction areas and to ensure that all genetic material and all potential reproduction habitats are in use. For the genetic resources of hatchery stocks to become a viable component of salmon evolution in the Baltic Sea, they need to be reintroduced into the wild as naturally reproductive stocks.

To be able to retain the upper genetic structure above stock level, upper hierarchical levels need to be considered in conservation strategies. The prioritized goal of conserving viability by using large population sizes, the evidence of lost variability because of small population sizes, and the observed isolation-by-distance pattern all emphasize the need to use larger units or certain stock groups as genetic management units in addition to the usual stock level.

The two goals of the maintenance of genetic diversity, keeping population sizes large and conserving the observed stock structure to the greatest extent possible, can be combined by constructing the conservation strategy from the top of the hierarchy instead of from the individual stock level. The population sizes maintained within units will limit the level to which it is possible to proceed in splitting the structure. As the two phylogeographic lineages still have relatively many stocks and sufficient variability for adaptation, at least these two large genetic management units can be formed. Separate strategies are needed to ensure maintenance of genetic diversity within the lineages; gene flow between them should be avoided. Similarly, if the stocks from the Gulf of Finland and the Eastern Main Basin can be considered to manage by themselves, a further distinction can be made and three management units formed. The establishing of management units depends basically on genetic differentiation, but information is also needed on potential reproduction areas, stock numbers, smolt production levels, geographical location of stocks, survival risks, fishing pressure, and possible differences in quantitative traits such as migration behaviour (Allendorf et al. 1997).

In principle, the same hierarchy as in the genetic structure should regulate the order of gene flow levels in the management strategy. Controlled gene flow should be allowed to maintain diversity between similar stocks. The position of a stock in a stock hierarchy can also be used as a weight value in the assessment of the conservation values for the stocks.

Treating the sea areas (Gulf of Bothnia, Gulf of Finland, Main Basin) separately would also be sensible for fisheries management. Each of the main sea areas has its own problems with its salmon stocks due to differences in reproduction potentials, genetic structure, migration routes, and fishing patterns. Thus, long-term strategies are needed to create solutions for each area separately, taking the local potential for maintaining naturally reproductive stocks into account.

Acknowledgements

We are very grateful to Dr. Risto Väinölä, Prof. Pekka Pamilo, Dr. Michael M. Hansen, Dr. Sakari Kuikka, and Dr. Einar E. Nielsen for their valuable comments. Gillian Häkli corrected the English. This work is part of the Finnish Biodiversity Research Program FIBRE.

References

Alalammi, P. (*Editor*). 1992. Atlas of Finland. Geology. National Board of Survey, Geographical Society of Finland, Helsinki, Finland. pp. 123–126.

Allendorf, F.W., Bayles, D., Bottom, D.L., Currens, K.P., Frissell, C.A., Hankin, D., Lichatowich, J.A., Nehlsen, W., Trotter, P.C., and Williams, T.H. 1997. Prioritizing Pacific salmon stocks for conservation. Conserv. Biol. **11**: 140–152.

Anatsky, S.Ju., and Dirin, D.K. 1997. Present status of Baltic salmon in Russia. Part 1. *In* Interactions between salmon culture and wild stocks of Atlantic salmon: the scientific and management issues. *Edited by* A.F. Youngson, L.P. Hansen, and M.L. Windsor. Norwegian Institute for Nature Research, Trondheim, Norway. pp. 90–96.

Anonymous. 1996. Report of the Baltic Salmon and Trout Assessment Working Group. ICES CM1996/Assess: 12.

Bakke, T.A., Jansen, P.A., and Hansen, L.P. 1990. Differences in the host resistance of Atlantic salmon, *Salmo salar* L., stocks to the monogenean *Gyrodactylus salaris* Malmberg, 1957. J. Fish Biol. **37**: 577–587.

Blanco, G., Sanchez, J.A., Vazquesz, E., Rubio, J., and Utter, F.M. 1992. Genetic differentiation among natural European populations of Atlantic salmon, *Salmo salar* L., from drainages of the Atlantic Ocean. Anim. Genet. **23**: 11–18.

© 1999 NRC Canada

Bourke, E.A., Coughlan, J., Jansson, H., Galvin, P., and Cross, T.F. 1997. Allozyme variation in populations of Atlantic salmon located throughout Europe: diversity that could be compromised by introduction of reared fish. ICES J. Mar. Sci. **54**: 974–985.

Chakraborty, R., and Leimar, O. 1987. Genetic variation within a subdivided population. *In* Population genetics and fishery management. *Edited by* N. Ryman and F. Utter. Washington Sea Grant Program. University of Washington Press, Seattle, Wash. pp. 89–120.

Clayton, J.W., and Tretiak, D.N. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. **29**: 1169–1172.

Cross, T.F., Galvin, P., and McGinnity, P. 1998. Genetic considerations in stocking Atlantic salmon, *Salmo salar*. *In* Stocking and introduction of fish. *Edited by* I.G. Cowx. Fishing News Books, Hull International Fisheries Institute, University of Hull, Hull, U.K. pp. 355–370.

Donner, J. 1995. The Quaternary history of Scandinavia. Cambridge University Press, New York.

Fontaine, P.-M., Dodson, J.J., Bernatchez, L., and Slettan, A. 1997. A genetic test of metapopulation structure in Atlantic salmon (*Salmo salar*) using microsatellites. Can. J. Fish. Aquat. Sci. **54**: 2434–2442.

Forstén, A., and Alhonen, P. 1975. The subfossil seals of Finland and their relation to the history of Baltic Sea. Boreas (Oslo), **4**: 143–155.

Kazakov, R.V., and Titov, S.F. 1993. Population genetics of salmon, *Salmo salar* L., in northern Russia. Aquacult. Fish. Manage. **24**: 495–506.

Kimura, M. 1953. "Stepping stone" model of population. Natl. Inst. Genet. (Mishima) Annu. Rep. **3**: 62–63.

Kimura, M., and Weiss, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics, **49**: 561–576.

Koljonen, M.-L. 1989. Electrophoretically detectable genetic variation in natural and hatchery stocks of Atlantic salmon in Finland. Hereditas, **110**: 23–35.

Koljonen, M.-L., and McKinnell, S. 1996. Assessing seasonal changes in stock composition of Atlantic salmon catches in the Baltic Sea with genetic stock identification. J. Fish Biol. **49**: 998–1018.

Koljonen, M.-L., and Pella, J.J. 1997. The advantage of using smolt age with allozymes for assessing wild stock contributions to Atlantic salmon catches in the Baltic Sea. ICES J. Mar. Sci. **54**: 1015–1030.

McCommas, S.A., and Bryant, E.H. 1990. Loss of electrophoretic variation in serially bottlenecked populations. Heredity, **64**: 315–321.

McConnell, S.K.J., Ruzzante, D.E., O'Reilly, P.T., and Hamilton, L. 1997. Microsatellite loci reveal highly significant genetic differentiation among Atlantic salmon (*Salmo salar* L.) stocks from the east coast of Canada. Mol. Ecol. **6**: 1075–1089.

Nei, M. 1977. *F*-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. **41**: 225–233.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and Takezaki, N. 1994. Estimation of genetic distance and phylogenetic trees from DNA analysis. Proc. 5th World Congr. Genet. Appl. Livestock Prod. **21**: 405–412.

Nei, M., Tajima, F., and Tateno, Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. **19**: 153–170.

Nielsen, E.E., Hansen, M.M., and Loeschke, V. 1999. Genetic variation in time and space: microsatellite analyses of extinct and extant populations of Atlantic salmon. Evolution, **53**: 261–268.

Ota, T. 1993. DISPAN: genetic distance and phylogenetic analysis. Institute of Molecular Evolutionary Genetics, Pennsylvania State University, 328 Mueller Laboratory, University Park, PA 16802, U.S.A.

Raymond, M., and Rousset, F. 1995*a*. An exact test for population differentiation. Evolution, **49**: 1280–1283.

Raymond, M., and Rousset, F. 1995*b*. GENEPOP (version 1.2): population genetics software for exact test and ecumenicism. J. Hered. **86**: 248–249.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation-by-distance. Genetics, **142**: 1357–1362.

Ryman, N. 1983. Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture, **33**: 1–21.

Ryman, N., Utter, F., and Laikre, L. 1995. Protection of intraspecific biodiversity of exploited fishes. Rev. Fish Biol. Fish. **5**: 417–446.

Saarnisto, M. 1970. The Late Weichselian and Flandrian history of the Saimaa Lake complex. Soc. Sci. Fenn. Comment. Physico-Math. **37**.

Saarnisto, M., Grönlund, T., and Ekman, I. 1995. Lateglacial of Lake Onega — contribution to the history of the Eastern Baltic Sea. Quat. Int. **27**: 111–120.

Saitou, N., and Nei, M. 1987. The neighbor joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.

Segerstråle, S.G. 1976. Immigration of glacial relicts into northern Europe. Boreas (Oslo), **5**: 1–5.

Slatkin, M. 1993. Isolation-by-distance in equilibrium and non-equilibrium populations. Evolution, **47**: 264–279.

Ståhl, G. 1983. Differences in the amount and distribution of genetic variation between natural populations and hatchery stocks of Atlantic salmon. Aquaculture, **33**: 23–32.

Ståhl, G. 1987. Genetic population structure of Atlantic salmon. *In* Population genetics and fishery management. *Edited by* N. Ryman and F. Utter. Washington Sea Grant Program. University of Washington Press, Seattle, Wash. pp. 121–141.

Swofford, D.L., and Selander, R.B. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**: 281–283.

Takezaki, N., and Nei, M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics, **144**: 389–399.

United Nations. 1992. Convention on biological diversity: 15 June 1992. United Nations Conference on Environment and Development, P.O. Box 80, CH-1231 Conches, Switzerland.

Varvio-Aho, S., and Pamilo, P. 1980. A new buffer system with wide applicability. Isozyme Bull. **13**: 114.

Waples, R. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. Mar. Fish. Rev. **53**: 11–22.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Wright, S. 1943. Isolation-by-distance. Genetics, **28**: 139–156.

© 1999 NRC Canada

Percentage land use in the watershed determines the water and sediment quality of 22 marshes in the Great Lakes basin

Barb Crosbie and Patricia Chow-Fraser

Abstract: Data from 22 Ontario marshes were used to test the hypothesis that distribution of forested, agricultural, and urban land in the watershed determines the water and sediment quality of Great Lakes wetlands. The first three components of the principal components analysis explained 82% of the overall variation. PC1 ordinated wetlands along a trophic gradient; species richness of submergent vegetation decreased with PC1 scores. PC2 reflected the content of inorganic solids and phosphorus in sediment and the ionic strength of the water. Both PC1 and PC2 scores were positively correlated with percent agricultural land, whereas PC1 scores were negatively correlated with forested land. Correlation between PC1 and agricultural land improved when best-management practices were considered. Accounting for common carp (*Cyprinus carpio*) disturbance did not confound the relationship between land use and water quality. PC3, driven by soluble reactive phosphorus and nitrate nitrogen concentration in the water, was not correlated with land use. Concentrations of polycyclic aromatic hydrocarbons and Metolachlor were correlated with urban and agricultural land, respectively, and may be useful as land use surrogates. Watershed management favouring the retention of forested land, or creation of buffer strips to trap agricultural runoff in the drainage basin, should help maintain aquatic plant diversity in coastal wetlands.

Résumé : Nous avons utilisé les données de 22 marais de l'Ontario afin de vérifier l'hypothèse selon laquelle la qualité de l'eau et des sédiments des milieux humides des Grands Lacs dépend de la répartition des terres boisées, agricoles et urbaines dans le bassin hydrographique. Les trois premières composantes de l'analyse des composantes principales expliquaient 82 % de la variation totale. La composante principale 1 (CP1) plaçait en ordonnée les milieux humides le long d'un gradient trophique; la richesse des espèces végétales partiellement submergées diminuait avec les valeurs de la CP1. La CP2 correspondait à la teneur en matières solides inorganiques et en phosphore des sédiments et à la force ionique de l'eau. Les valeurs de la CP1 et de la CP2 présentaient une corrélation positive avec le pourcentage de terres agricoles, tandis que les valeurs de la CP1 présentaient une corrélation négative avec les terres boisées. La corrélation entre la CP1 et les terres agricoles était meilleure lorsque l'on considérait les meilleures pratiques de gestion. Le fait de prendre en compte la perturbation de la carpe n'a pas troublé le rapport entre l'utilisation des terres et la qualité de l'eau. La CP3, commandée par la concentration de phosphore réactif soluble et de l'azote des nitrates dans l'eau, n'était pas corrélée à l'utilisation des terres. Les concentrations d'hydrocarbures aromatiques polycycliques et de métolachlore étaient corrélées respectivement avec les terres urbaines et les terres agricoles, et peuvent être utiles comme substituts de l'utilisation des terres. La gestion des bassins hydrographiques favorisant la conservation des terres boisées, ou la création de bandes tampons pour piéger les eaux de lessivage des terres cultivées dans le bassin hydrographique, devrait permettre le maintien de la diversité des plantes aquatiques dans les milieux humides côtiers.

[Traduit par la Rédaction]

Introduction

It has been recognized for over two decades that different amounts and types of nutrients exported from agricultural, urbanized, and forested landscapes have a direct effect on the trophic quality of downstream rivers and lakes (Beaulac and Reckhow 1982; Peterjohn and Correll 1984; Nelson et al. 1996). These studies have focussed on phosphorus and nitrogen, since they are known to limit the growth of algae in lakes. Using export coefficients associated with different land use categories, investigators have been able to predict or hindcast changes in lake water nutrient concentrations in response to projected or past land use alterations (e.g., Frink 1991; Johnes et al. 1996; Mattikalli and Richards 1996).

By comparison, the impact of different land uses on the water quality of wetlands has seldom been documented, and seldom for more than one watershed at a time (Ehrenfeld 1983; Smith et al. 1991). The tendency to use nutrient levels alone to indicate trophic quality may be problematic for wetlands, since both submersed aquatic vegetation and algae are primary producers, and macrophytes are much more limited by light availability (Hough et al. 1989; Lougheed et al. 1998; Chow-Fraser 1998) and substrate type (Barko et al.

Received May 11, 1998. Accepted June 23, 1999.
J14576

B. Crosbie and P. Chow-Fraser.[1] Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada.

[1]Author to whom all correspondence should be addressed.
e-mail: chowfras@mcmaster.ca

© 1999 NRC Canada

Table 1. Summary of general descriptions of wetlands in the study.

Wetland	Latitude, longitude	Wetland size (km^2)	Watershed size (km^2)	Wetland type	Hydrologic character	Common carp disturbance
1. 15 Mile Creek Marsh	43°10′00″, 79°19′00″	0.31	63	Riverine	No flow	Yes
2. Big Creek Marsh	42°57′20″, 80°26′50″	0.61	200	Riverine	No flow	Yes
3. Centreville Creek Marsh	43°54′04″, 79°50′00″	0.13	35	Riverine	Observed flow	
4. Christie Lake Marsh	44°47′00″, 76°28′00″	1.13	66	Lacustrine	No flow	
5. Cootes Paradise Marsh	43°16′00″, 79°55′00″	2.5	268	Riverine	No flow	Yes
6. Harris Lake Marsh	45°42′00″, 80°82′00″	20	>1000	Lacustrine	No flow	
7. Hay Bay Marsh	44°10′30″, 76°55′30″	13.33	225	Riverine	No flow	Yes
8. Holiday Marsh	42°02′05″, 83°03′00″	11.77	64	Riverine	No flow	Yes
9. Humber River Marsh	43°38′00″, 79°29′00″	0.26	667	Riverine	No flow	Yes
10. Joe's Lake Marsh	45°08′00″, 76°41′00″	2.04	268	Lacustrine	No flow	
11. Jordan Harbour Marsh	43°11′00″, 79°23′00″	1.72	311	Riverine	No flow	Yes
12. Martindale Pond Marsh	43°10′07″, 79°16′00″	0.55	29	Riverine	No flow	Yes
13. Presqu'ile Marsh	44°00′00″, 77°43′00″	9.92	22.2	Lacustrine	No flow	
14. Sawguin Marsh	44°06′00″, 77°23′00″	20.93	91	Lacustrine	No flow	Yes
15. Second Marsh	43°52′00″, 79°51′00″	1.06	85	Lacustrine	No flow	Yes
16. Shebeshekong River Marsh	45°24′30″, 80°19′00″	1.08	107	Riverine	Moderate flow	
17. Stump Lake Marsh	44°56′48″, 76°38′12″	0.93	1060	Lacustrine	No flow	
18. Sutton Pond Marsh	42°50′00″, 80°18′00″	0.10	85	Riverine	No flow	Yes
19. Tay River Marsh	44°52′45″, 76°10′30″	5.53	669	Riverine	No flow	
20. Tobies Bay Marsh	44°51′00″, 79°47′00″	1.94	9	Riverine	No flow	
21. Turkey Creek Marsh	42°14′08″, 83°05′07″	0.32	5	Riverine	No flow	Yes
22. Waterford Pond Marsh	42°56′10″, 80°18′45″	2.39	71	Lacustrine	No flow	Yes

1991) than by nutrient concentration in the water column. Hence, studies that do not consider nonalgal turbidity and sediment quality may have limited value for understanding land use impacts on the trophic quality of wetland communities. In the Great Lakes basin, in-marsh effects such as sediment resuspension by the common carp (*Cyprinus carpio*) are also known to contribute significantly to water turbidity (e.g., Whillans 1996; Lougheed et al. 1998; Chow-Fraser 1998) and may confound any external effects from non-point-sources in the watershed.

This study was initiated to determine the impact of land use on water and sediment quality of coastal marshes in the Great Lakes basin because these wetlands have been lost at an alarmingly high rate following European settlement (Whillans 1982). This loss has occurred in spite of their great value in water storage and groundwater recharge, flood prevention, and sediment and nutrient filtration and their vital ecological role in maintaining biodiversity and providing habitat for plants, birds, mammals, fish, and invertebrates (Maynard and Wilcox 1996).

Until recent decades, most of these wetlands were lost through conversion to agricultural land and (or) use for urban development. At present, however, wetlands are more at risk from non-point-source pollution and in-marsh stresses such as carp disturbance and water level regulation than from dredging, infilling, or draining (Keddy and Reznicek 1986; Maynard and Wilcox 1996). If governments are to seriously devise strategies to protect and restore the remaining wetlands, scientists must begin to provide some basic understanding of factors that impair water quality of wetlands, since deterioration in water quality has been consistently linked to marsh degradation (Crowder and Painter 1991; Chow-Fraser et al. 1998).

In this paper, we present seldom-documented water and sediment quality information for 22 Ontario marshes in the Great Lakes basin. We used principal components analysis (PCA) to create a multidimentional index of trophic quality for each wetland to test the relationship between this index and the relative distribution of forested, agricultural, and urban land in respective watersheds. We also simultaneously consider the impact of land use both in the presence and absence of carp disturbance to evaluate the relative importance of the latter on water quality in wetlands. Since current land use information is often difficult to acquire, we investigate the potential for using organic contaminants specific to agricultural or urban landscapes to predict land use. The level of polycyclic aromatic hydrocarbons (PAHs) in wetland sediment was used to predict the proportion of urbanized land in our watersheds, since PAHs are common pollutants associated with roadway runoff (Maltby et al. 1995). As a predictor of the proportion of agricultural land, we used the concentration of Metolachlor in upstream creeks and rivers that feed into our wetlands, since this herbicide is typically associated with agricultural runoff (Gaynor et al. 1995). Finally, we relate water quality impairment associated with basin development to changes in the species richness of submersed aquatic vegetation and thus provide a direct link between land use and one possible measure of ecosystem health in coastal wetlands.

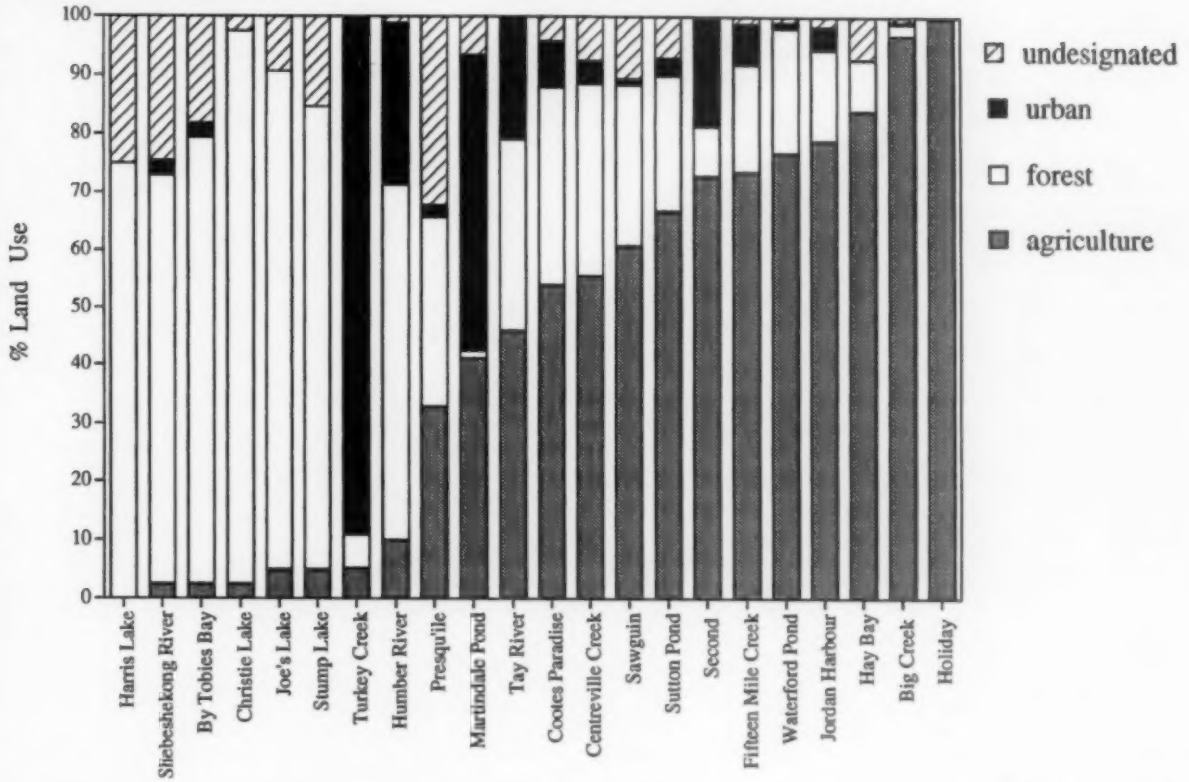
Materials and methods

Study sites

Twenty-two wetlands, covering a broad spectrum from highly disturbed to pristine conditions, were sampled for a suite of water quality variables and vegetation in 1995 and 1996 (Table 1). Wet-

© 1999 NRC Canada

Fig. 1. Relative distribution of different land uses in the watersheds of the 22 wetlands in this study.



land sites were chosen based on the amount of urban, agricultural, and forested land in their watershed to ensure a sufficient gradient of disturbance (Fig. 1). A survey of the submergent plant community was conducted in each wetland at the time of sampling, as described in Lougheed et al. (1998). All emergent and submergent aquatic vegetation was identified in the field to taxa and samples of representative species were collected and identified in the laboratory to species. It was not our intent to conduct a comprehensive plant survey of each wetland but to choose a site within the wetland that had a structurally diverse plant community consisting, where possible, of emergent, submergent, and floating taxa interspersed with open water. Hence, only plant taxa in the vicinity of the area sampled for water and sediment were critically evaluated. This area totalled a minimum of 100 m^2 in each of the 22 wetlands, but in some cases more than 400 m^2. Emergent taxa encountered in this survey included species of *Typha*, *Scirpus*, *Pontederia*, *Sparganium*, *Decodon*, and *Sagittaria*; submergent taxa included species of *Vallisneria*, *Potamogeton*, *Vallisneria*, *Utricularia*, *Ceratophylum*, *Najas*, and *Elodea* (Crosbie 1997).

Field collection and observations

At each site, we collected water and sediment samples twice during the summer in either 1995 or 1996. Water and sediment samples were collected from an open-water location at least 3 m away from a stand of emergent vegetation, except in the case of Centreville Creek Marsh where the sampling station was restricted to a vegetated tributary less than 1 m wide. In situ measurements of pH and specific conductance were recorded with a Hydrolab H20 sonde equipped with a Scout2 monitor. The Hydrolab was calibrated for conductivity, pH, and dissolved oxygen with known standards prior to each sampling trip. Turbidity values were collected with a Hach turbidimeter (model 2100P) that was calibrated biweekly with known standards. Secchi depth transparencies were recorded in triplicate with a 20-cm disc from the shaded side of the sampling vessel. To control for differences due to weather conditions, we excluded data collected during rain events. Therefore, all samples were collected following at least 72 h of dry weather. The presence of carp at each site was verified visually during our sampling and (or) from wetland evaluation reports by the Ontario Ministry of Natural Resources. These data are purely qualitative, as they represent only the presence–absence of carp, but we know from other studies that the presence of carp can alter water clarity over a wide range of fish biomass (Lougheed et al. 1998).

Nutrient, chlorophyll, and suspended solids analyses

On each sampling occasion, we collected water samples from the middle of the water column with a horizontal 1-L Van Dorn bottle. Water samples for nutrient analyses were collected in clean, acid-washed Nalgene bottles and stored at 5°C until our return to the laboratory. In the laboratory, soluble reactive phosphorus (SRP) samples were filtered immediately through a 0.45-μm GF/C filter and kept frozen until analysis. Whole-water samples for total phosphorus (TP) and total nitrate-nitrogen (NO_3-N) were also frozen until analysis. Whole-water samples for total ammonia-nitrogen (NH_4-N) were acidified to pH 2.0 and stored at 5°C until analysis. All samples were processed usually within 6 h of collection, and all analyses were conducted within 3 months of collection.

Prior to analysis, a standard curve for phosphorus was generated from standards of known values. TP and SRP samples were analyzed in triplicate according to Murphy and Riley (1962), with TP

© 1999 NRC Canada

samples digested according to standard methods (American Public Health Association 1992) prior to phosphorus determination. All absorbance readings were read with a Milton Roy Spec 301 spectrophotometer. We followed EPA-approved Hach methods and used Hach reagents (Hach Company 1989) to measure NH_4-N and NO_3-N concentrations in duplicate; absorbances were read with a Hach DR2000 spectrophotometer and converted to concentrations using regularly calibrated standard curves. The accuracy of all nutrient analyses were within 10% of actual concentrations.

Whole-water samples for chlorophyll *a* (CHL) analysis were collected in clean, opaque Nalgene bottles and stored at 5°C until our return to the laboratory where they were immediately filtered through 0.45-μm GF/C filters. Filters were folded, wrapped in aluminum foil, and kept frozen for up to 3 months before they were analyzed. CHL samples were extracted in 90% reagent-grade acetone (in the freezer), acidified, and analyzed for CHL pre- and post-acidification with a Spec 301 spectrophotometer. Equation 1 of Speziale et al. (1984) was used to calculate CHL concentrations, and all values reported here have been corrected for phaeopigments.

Samples for total suspended solids (TSS) were first filtered through preweighed GF/C filters and then frozen for less than 3 months. They were dried at 100°C for 1 h, placed in a desiccator for 1 h, and then weighed to the nearest 0.0001 g with an OHAUS AS 120 balance. Filters were subsequently combusted at 550°C for 20 min and desiccated for 1 h to determine ash-free weights or total inorganic suspended solids (TISS). Total organic suspended solids (TOSS) was calculated as the difference between TSS and TISS.

Phosphorus and inorganic content of sediment

Sediment samples were collected from the top 5 cm of sediment with either an Eckman grab sampler or a 5-cm-diameter Plexiglas tube equipped with a plunger. Sediment samples were dried at 100°C, ground to achieve homogeneity, and fired at 550°C in a muffle furnace to calculate the amount of inorganic material contained within the sediment. The TP in the sediment was analyzed according to Andersen (1976). Between 0.15 and 0.2 g of this material was weighed and boiled in 25 mL of 1 M HCl for 15 min and the extract was diluted to 100 mL. After neutralization with 10 M NaOH, a 20-mL aliquot was analyzed for TP content of the sediment according to the protocol for phosphorus analyses (above).

Analyses of trace organics by immunoassay techniques

PAHs and Metolachlor are organic contaminants that are commonly present in disturbed environments. Their presence can be detected by conventional gas chromatography (GC) methods that are expensive and labour-intensive. Recently, immunoassay methods have been used as a screening tool, as they are more cost-effective and less time-consuming than GC methods. Immunoassay techniques and conventional GC/mass spectrometry methods have been compared in the literature and were found to yield comparable data for both PAHs and other herbicides in laboratory and field samples (Zaruk et al. 1995). Direct comparisons between GC and immunoassay results for a subset of these wetlands show that samples measured by immunoassays tend to overestimate contaminant concentrations provided by GC methods; however, since this error is consistent throughout the range of concentrations tested, valid comparisons can be made among data obtained by the same technique (Crosbie 1997). Since immunoassay results have compared favourably with those of traditional GC methods, we chose to use the less expensive method in this study with 4% of the samples analyzed by GC methods as a confirmatory measure.

PAH

Samples were collected from 10 of the 22 wetlands, sampled in 1995, to determine the PAH content in sediment. Analyses were performed on these select wetlands due to budget and time constraints. The top 5 cm of sediment was collected using a Plexiglas tube (5 cm inside diameter) equipped with a plunger. These samples were kept at 5°C until analyses and placed in a dehydrator for 24 h at 35°C to remove excess water. Subsamples were stored in a desiccator until analysis by immunoassay. We used the Millipore EnviroGard PAH in Soil Plate Kit (Millipore Intertech) to extract and analyze for total PAHs (range from 1.0 to 10 $ng \cdot g^{-1}$ in soils). The enzyme-linked immunoassay technique utilizes polyclonal antibodies coated to the walls of microwells in test kits and an enzyme conjugate that competes for binding sites with the contaminant of interest in the collected sample. The amount of the target compounds in the sample is thus inversely proportional to the amount of colour produced by the reaction. The coloured products were measured spectrophotometrically on a microplate reader fitted with a 450-nm filter. Duplicates, spikes, and blanks were run on the kit to measure variation, recovery, and contamination, as recommended by the manufacturer.

Metolachlor concentration in water

Surface samples were collected in washed and solvent rinsed glassware with teflon- or foil-lined lids from 14 different marshes during June and July in 1996. They were stored in the dark in amber bottles at 5°C until analysis by immunoassay. We used Quantix Metolachlor 1.0 immunoassay kits (Idetek Inc., Sunnyvale, Calif.) to measure the Metolachlor concentrations and followed instructions recommended by the manufacturer. Samples were measured spectrophotometrically at 650 nm, and concentrations were determined from a calibrated standard curve.

Land use delineation

Relevant maps from the 1 : 50 000 National Topographic Series were used to delineate the wetland watersheds, except for those in the Niagara region, where 1 : 25 000 land use maps that included spot elevations were used to delineate the watersheds. The watershed was delineated from contour lines of the highest elevation and (or) equidistant points between two tributaries of adjacent watersheds. Forested, agricultural, and urban land within the watersheds was determined from Ontario's Agricultural Land Use Series maps (majority being current to the mid-1980s) and supplemented by information from aerial surveys taken during 1996 in cases where land use maps were incomplete (i.e., Cootes Paradise Marsh, Christie Lake Marsh, Joe's Lake Marsh, Stump Lake Marsh, Hay Bay Marsh, Sawguin Marsh, Tay River Marsh, and Presqu'ile Marsh). Such aerial surveys confirmed that the land use data available were accurate for the purposes of this comparative study. Triplicate planimetry measurements were used to quantify the size of the wetland, watershed, and each of the three land use types. Where applicable, presence of buffer strips surrounding wetlands was also confirmed from aerial surveys and land use maps.

Statistical analysis

All statistical analyses were performed using SAS Jmp software for the Macintosh (SAS Institute Inc., Cary, N.C.). Statistical tests included paired *t* tests, correlation analysis, PCA, and general linear regression analysis. To achieve normality, data were either log transformed or arcsine transformed to normalize the variances. We used standardized residuals to identify outliers in the linear regression analyses, since they can have a strong influence on estimates of the fitted parameter. This exercise allowed us to eliminate Big Creek and Holiday Marsh (wetlands 2 and 8, respectively, in Table 1) from the regression of PC1 scores against percent agricultural land.

Results

The size of wetlands varied, ranging from 0.10 to over

© 1999 NRC Canada

20 km^2, with corresponding watersheds ranging from very small (5 km^2) to extremely large (1060 km^2) (Table 1). In addition, these sites represented both riverine and lacustrine wetlands. Water flow through the wetland at the sampling site was generally very slow (or not observable), except for Centreville Creek Marsh where the observed water flow was greater than 2 $m \cdot s^{-1}$. This riverine wetland was also unique in that it was heavily vegetated with emergent plants only, with no submergent plants or open-water areas that were found in all of the other 21 wetlands. Slightly more than half of these wetlands are known to be stressed by carp disturbance, although the relative importance of this stressor was not evaluated quantitatively (e.g., biomass, mean density). The fact that there are different types of wetlands covering a large range of ecological conditions should strengthen the utility of this study and make our conclusions more generally applicable.

PCA

We first determined if data collected from the two sampling trips in each year could be combined. Since we found no significant differences between sampling dates for any of the 14 variables (paired t tests; $P > 0.05$), we merged data for the two trips and calculated averages for each variable (Table 2). PCA was subsequently used to determine the best explanatory variables in the water quality and sediment quality data set. The first three axes together explained 82% of the overall variation in the data set (Table 3). PC1, which accounted for over half of the variation, was positively and highly correlated with environmental variables that were essentially measures of particulate content in the water column (Fig. 2). These included water turbidity and concentrations of TSS, TP, and planktonic CHL. TP and total NH_4-N were also significantly correlated with PC1 scores, even though it is not a reflection of the suspended particles in the water. PC2 explained 18% of the variance and was positively correlated with the inorganic content of the sediment but was negatively correlated with its TP content. Wetlands that had high positive PC2 scores tended to have higher pH and specific conductance. PC3, which only accounted for 13% of the variance, was negatively correlated with SRP concentration but positively correlated with total NO_3-N concentration in the water column.

Taken together, the PCA scores reflected the degree of water and sediment quality impairment in the wetland: sites with high positive scores for all three components were turbid systems, enriched with phosphorus and nitrogen, with correspondingly high CHL in the water column, and lacking phosphorus and organic content in the sediment. By contrast, wetlands associated with low and negative scores were characterized by clear water, low nutrient concentrations, and correspondingly low CHL, with sediments rich in both organic content and phosphorus (Fig. 2).

Submergent vegetation

In a concurrent study, Lougheed et al. (1998) surveyed the number of submergent plant species in each of these wetlands. We found an inverse relationship between PC1 scores and the number of species of submersed aquatic vegetation (Fig. 3). Marshes with low water turbidity and nutrients supported a more species-rich community of submergent plants. Centreville Creek Marsh (wetland 3, Table 1), which did not support submergent plants, was excluded from this analysis. By comparison, the species richness of submergent plants did not vary with PC2 scores; hence, neither sediment fertility nor organic content appears to be a determinant of submergent diversity in these wetlands.

Land use and water and sediment quality

To test the hypothesis that the distribution of different land use types in the watershed affected the trophic quality of wetlands, we regressed scores of the first three principal components against the proportion of agricultural, forested, and urban land. We found three significant relationships: (*i*) PC1 scores varied inversely with the proportion of forested land (PC1 score = –4.415 arcsin FOR + 1.325; r^2 = 0.40, $P < 0.001$), (*ii*) PC1 scores varied directly with the proportion of agricultural land (PC1 score = 2.15 arcsin AGR – 2.43; $r^2 = 0.43$, $P = 0.0017$; Fig. 4*a*), and (*iii*) PC2 scores were negatively related to the proportion of forested land (PC2 score = –2.888 arcsin FOR + 0.471; $r^2 = 0.42$, $P < 0.001$). Accordingly, wetlands whose watersheds were dominated by agricultural areas tended to be turbid and nutrient rich, while those within forested landscapes were clear and nutrient poor. Unfortunately, owing to the small number of urban wetlands in this study, the absence of a significant relationship between principal components scores and percentage urban land is inconclusive.

Peterjohn and Correll (1984) demonstrated that forested buffer strips can reduce the nitrogen and phosphorus loads by up to 70% if they are located near the edge of a riparian system. We accounted for the ameliorating effects of such buffer strips within agricultural areas by applying a conservative reduction factor of 0.25 to sites that had forested buffer zones along one or more of their tributaries (Centreville Creek Marsh (wetland 3), Hay Bay Marsh (wetland 7), Sawguin Marsh (wetland 14), and Waterford Marsh (wetland 22)) and a factor of 0.50 to sites that had a large proportion of forested land (Presqu'ile Marsh (wetland 13) and Tay River Marsh (wetland 19)). When we compared the unmodified index of agricultural land with the modified index, we found that regression of PC1 scores against the latter improved significantly (r^2 increased from 0.43 to 0.68, respectively; Fig. 4). This comparison suggests that the existence of forested areas in agriculturally dominant watersheds may lead to measurable differences in the water quality of downstream wetlands.

Common carp disturbance

We had the opportunity to broadly evaluate the effect of carp disturbance on the trophic quality of wetlands as indicated by PC1 scores. Study sites that had been invaded by carp were well represented (solid squares in Fig. 4). Many of the wetlands known to be affected by carp disturbance were in fact associated with medium to high PC1 scores; however, there were at least two marshes (Hay Bay (wetland 7) and Sawguin (wetland 14)) that also had relatively low scores. Therefore, the presence of carp in the wetland had an inconsistent effect on water quality and did not confound the relationship between water quality and land use.

© 1999 NRC Canada

Table 2. Summary of key water quality and sediment quality data for wetlands identified by PCA.

Wetland	Turbitity (NTU)	Light extinction coefficient	TSS ($mg \cdot L^{-1}$)	TISS ($mg \cdot L^{-1}$)	TOSS ($mg \cdot L^{-1}$)	TP ($\mu g \cdot L^{-1}$)	SRP ($\mu g \cdot L^{-1}$)	CHL ($\mu g \cdot L^{-1}$)	TAN ($mg \cdot L^{-1}$)	TNN ($mg \cdot L^{-1}$)	Specific conductance ($\mu S \cdot cm^{-1}$)	pH	$Inorg_{sed}$ ($mg \cdot g^{-1}$)	TP_{sed} ($mg \cdot g^{-1}$)
1	80.7	7.60	124	112	12	345	20	24	0.195	0.450	741	7.5	0.94	0.72
2	4.1	2.47	11.2	5.2	6	76	9.2	7.3	0.061	1.760	554	7.6	0.81	0.60
3	3.8	2.45	4.6	1.5	3.1	66	13	2.6	0.083	0.050	599	7.6	0.64	1.41
4	2.6	2.37	8.8	4.2	4.6	31	2.8	6.3	0.026	0.080	156	6.7	0.59	1.47
5	59.0	6.14	51	37	14	191	24	24	0.355	0.262	715	7.3	0.82	0.82
6	1.8	2.31	3.8	1.0	2.8	81	4.4	2	0.355	0.050	39	6.0	0.44	1.92
7	3.0	2.40	3.7	1.3	2.4	62	22	7.7	0.066	0.046	552	6.7	0.68	0.57
8	265	19.94	248	160	88	770	24	247	0.296	1.419	601	7.5	0.80	0.66
9	67.8	6.74	57	37	20	214	11	29	0.221	0.140	735	7.5	0.95	0.55
10	1.6	2.30	5.4	2.7	2.7	23	2	6.0	0.082	0	215	6.9	0.41	2.00
11	44.0	5.14	42	30	12	360	88	61	0.094	0.221	618	8.1	0.93	0.69
12	44.1	5.15	75	50	25	339	116	45	0.160	0.466	452	7.1	0.92	0.75
13	1.5	2.29	4.8	0.7	4.1	54	1.9	2.4	0.024	0.030	336	7.1	0.79	0.99
14	4.1	2.47	6.5	1.8	4.7	76	15.4	15	0.115	0.174	390	6.9	0.28	1.99
15	14.8	3.19	24	15	9	407	107	67	0.035	0.433	865	7.8	0.71	1.01
16	6.1	2.6	9.5	4.2	5.3	69	1.5	6.1	0.063	0.335	49	6.1	0.81	0.41
17	2.1	2.3	9.3	6.2	3.1	42	3.6	5.7	0.060	0.004	190	6.5	0.24	2.17
18	13.4	3.09	42	30	12	80	2.6	18	0.067	1.733	529	8.2	0.94	0.39
19	1.4	2.29	6.2	3.2	3.0	50	6.6	4.6	0.049	0.065	195	7.2	0.26	2.04
20	2.8	2.38	7.4	1.0	6.4	139	2.9	22	0.040	0.294	100	6.3	0.36	1.03
21	11.7	2.98	20.8	14.0	7.8	61	12.4	21	0.041	0.048	479	8.3	0.82	0.88
22	23.0	3.74	52	29	23	129	7.3	25	0.265	0.450	465	7.8	0.75	0.85

Note: Values are averages of two sampling trips. TSS, total suspended solids; TISS, total inorganic suspended solids; TOSS, total organic suspended solids; TP, total phosphorus; SRP, soluble reactive phosphorus; CHL, chlorophyll *a*; TAN, total NH_4-N; TNN, total NO_3-N; $Inorg_{sed}$, proportion inorganic content in sediment; TP_{sed}, total phosphorus in sediment. Wetland numbers are given in Table 1.

© 1999 NRC Canada

Table 3. Correlation coefficients between principal components scores and environmental variables.

	Variance explained (%)	Environmental variable	*r*	*P*
PC1	51	Water turbidity	0.94	0.0001
		TSS	0.94	0.0001
		TOSS	0.93	0.0001
		TISS	0.91	0.0001
		TP	0.91	0.0001
		CHL	0.89	0.0001
		TAN	0.74	0.0001
PC2	18	$Inorg_{sed}$	0.84	0.0001
		TP_{sed}	−0.82	0.0001
		pH	0.68	0.0001
		Conductance	0.66	0.0001
PC3	13	SRP	−0.61	0.004
		TNN	0.60	0.004

Fig. 2. Plots of (*a*) PC2 scores and (*b*) PC3 scores versus PC1 scores. Numbers inside the squares are the number of submersed aquatic plant taxa encountered during our surveys. The data point corresponding to Centreville Creek Marsh (wetland 3) is labelled in each panel.

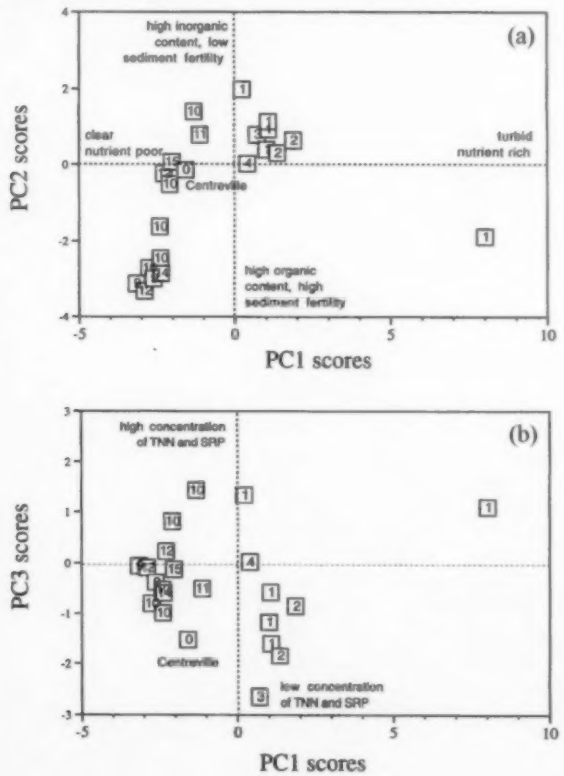
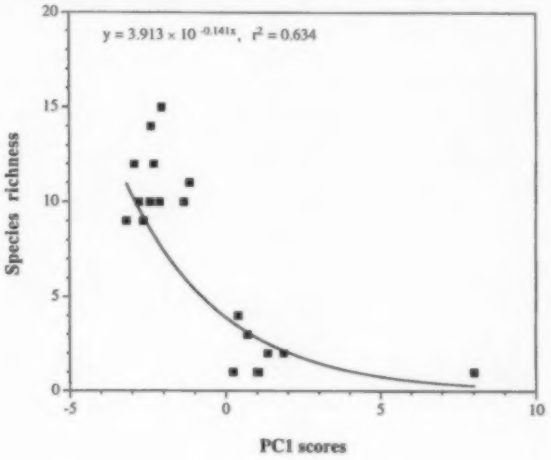


Fig. 3. Number of submersed aquatic plant taxa plotted against PC1 scores for each of the wetland. Centreville Creek Marsh (wetland 3) has been excluded from this analysis (see text).



Land use surrogates

We had some difficulty obtaining current land use information for all sites in this study. This, together with the large amount of time and labour required to calculate percent land use (especially in the absence of digitized land use information), seriously limited our ability to study wetlands on a basin-wide scale. Therefore, we explored the potential for using easily measured field parameters specific to agricultural or urban activities as land use surrogates for future investigations. The parameters that we chose to test were the level of total PAHs in wetland sediment and the concentration of Metolachlor in creek and river water feeding into the wetlands. We reasoned that PAHs are common pollutants associated with roadway runoff (Maltby et al. 1995) and should therefore be an effective indicator of percent urbanized land in watersheds. Metolachlor concentration was chosen to indicate percent agricultural land because it is a commonly used herbicide in Ontario and has been found consistently in agricultural runoff (Gaynor et al. 1995).

The total level of PAHs in the sediment significantly explained close to 70% of the variation in urbanized land, whereas $\log_{10}$-transformed Metolachlor concentration explained 80% of the variation in percent agricultural land (Fig. 5). Therefore, these measures of organic contaminants in sediment and water demonstrate their potential usefulness as land use surrogates. When we regressed PC1 against log-transformed Metolachlor concentrations, we found a highly significant relationship ($r^2 = 0.77$, $P = 0.0001$, $n = 16$) that confirmed the utility of this variable as a surrogate of percent agricultural land. Although these results are promising, the sample size in this study was very small, and more data need to be collected in the future to determine the general applicability of these results to other wetlands.

Discussion

There are few published studies that characterize the ecological relationships of Great Lakes coastal wetlands on a

© 1999 NRC Canada

Fig. 4. PC1 scores versus (*a*) arcsine-transformed percent agricultural land (AGR) in the watershed and (*b*) arcsine-transformed percent agricultural land (MOD AGR) after applying a reduction factor of 0.5 for wetlands that had large forested buffer zones (Presqu'ile Marsh (wetland 13) and Tay River Marsh (wetland 19)) and a factor of 0.25 to sites that only had forested strips along one or more of their tributaries (Centreville Creek Marsh (wetland 3), Hay Bay Marsh (wetland 7), Sawguin Marsh (wetland 14), and Waterford Marsh (wetland 22)). Least squares regression analysis did not include the outliers Big Creek (wetland 2) and Holiday Marsh (wetland 8). Solid and open squares correspond to wetlands that are accessible and inaccessible to common carp, respectively.

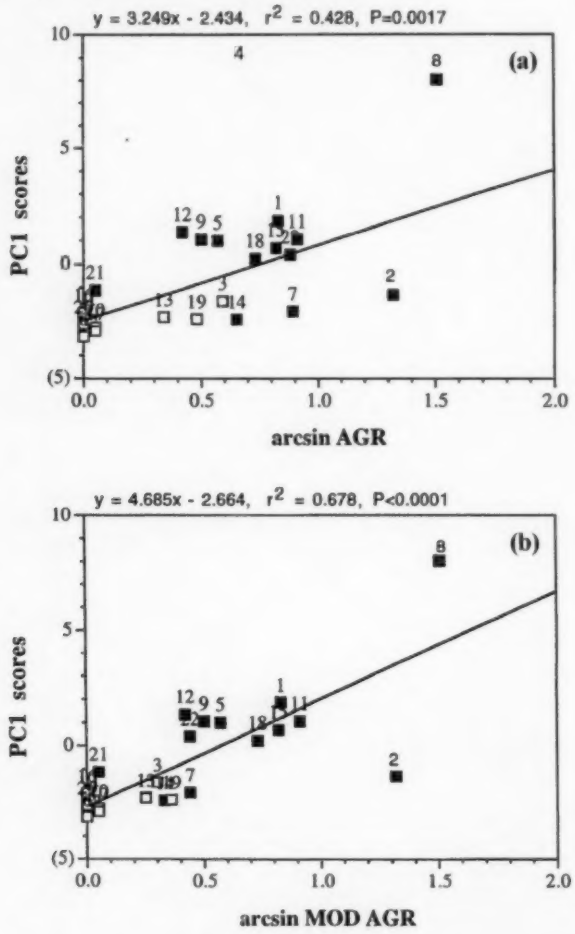
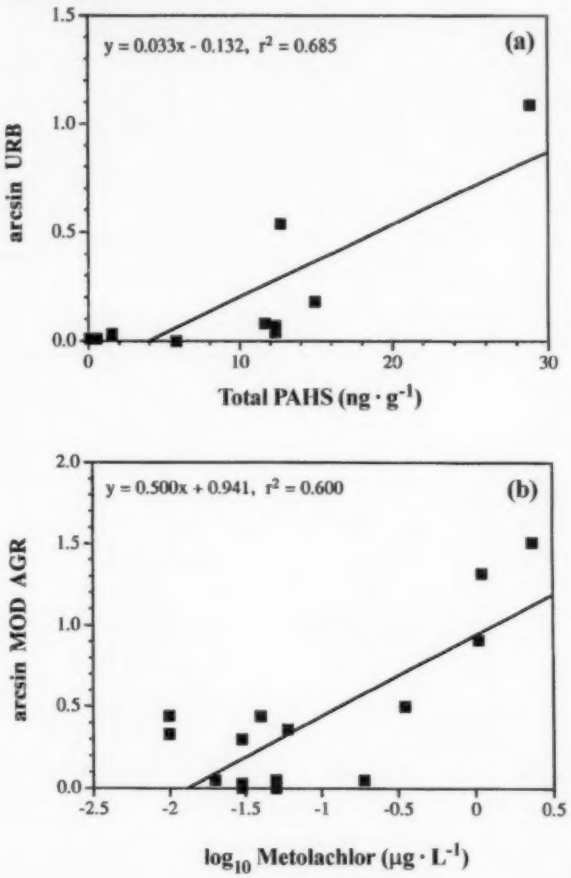


Fig. 5. (*a*) Arcsine-transformed percentage urbanized land (URB) versus total PAHs in wetland sediment and (*b*) arcsine-transformed percentage agricultural land (MOD AGR) modified to account for the ameliorating effects of buffer strips (see legend to Fig. 4) versus log-transformed Metolachlor concentration in the water.



basin-wide scale. A notable exception is Smith et al. (1991)'s study of 160 Canadian coastal wetlands located mainly in the lower lakes and the St. Lawrence River. They showed that patterns of variation in wetland and site types, soil composition, dissolved solids concentration, vegetation complexity, and the presence of rare flora and fauna could be attributed to the geology of different lake basins. Findlay and Houlahan (1997) found that the species richness of some mammal and herpetile taxa was directly related to the proportion of forested land within a 2-km radius of wetlands and that removal of forested land threatened the biodiversity of these wetlands. Our study shows that the biodiversity of aquatic plants is similarly affected by altered land use. Apart from these few studies, large-scale investigations that examine the impact of land use changes on Great Lakes wetlands are lacking, even though such indirect impacts may cause greater damage to wetlands than can direct impacts such as drainage and infilling (Keddy 1983).

Although we found no comparable studies from other geographic regions that focussed on freshwater coastal wetlands, there are a few published studies that examined the distribution of aquatic macrophytes along ecological or disturbance gradients in inland wetlands and lakes. Moore et al. (1989) showed that infertile wetlands in north-central Ontario had higher species richness and many more rare species than fertile wetlands. Our observations in these wetlands echo their opinion that infertile wetlands are more desirable for conservation. Current wetland evaluation systems do not gain an adequate measure of fertility, which ap-

© 1999 NRC Canada

pears to be essential in determining diversity. Toivonen and Huttunen (1995), who sampled 57 small lakes in southern Finland, also found that the distribution of submerged aquatic vegetation was principally determined by trophic state and water transparency. However, the highest species diversity of submergents was found in clear, mesoeutrophic lakes, which were intermediate in trophic state between oligotrophic, brown-water lakes (humic stained) and hypereutrophic lakes. Expansion of our data set to include bogs (humic stained) may have yielded a similar trend.

By contrast, Morgan and Philipp (1986), who worked on streams in the New Jersey Pine Barrens, found a slight increase in the number of aquatic macrophytes in nitrogen-enriched streams following four decades of agricultural and urban development. In addition, there was evidence of species replacement: the native flora that were tolerant of nitrogen-limited environments had become outcompeted by species normally found outside the Barrens that tolerated phosphorus-limited conditions. Thus, it is important to consider both the species richness and the species composition of wetland ecosystems when evaluating the possible impact of land use changes. The restricted sample size of wetlands in this study did not permit us to analyze changes in species composition with trophic quality, but this topic is being investigated elsewhere with a larger data set (V.L. Lougheed et al., unpublished data).

In our study, concentrations of phosphorus, nitrogen, and inorganic suspended solids in wetlands increased predictably as agriculture became the dominant land use in their respective watersheds. These results are entirely consistent with previous studies on stream ecosystems, where 50–80% of the variation in nutrient and suspended particulate concentrations was related to the amount of agricultural land in the watershed (Osborne and Wiley 1988; Johnson et al. 1997). Holiday Marsh (wetland 8 in Fig. 4) exemplified this damage from agricultural runoff to an extreme and is probably why it was revealed as an outlier in the analysis of residuals. It had a mean water turbidity of 265 NTU, compared with 80 NTU for Big Creek, the wetland with the next highest water turbidity. The low relief in the watershed is quite unique to the region, and consequently, the entire watershed is farmed (Fig. 1). The type of crops grown include corn, soybean, and winter wheat, which usually requires tilling and extensive use of fertilizer, the cumulative negative effects of which are amply evident in the extremely degraded state of Holiday Marsh today. Therefore, Holiday Marsh should only be considered a statistical outlier in this study, but should be viewed as representing the extreme in water turbidity for all coastal marshes. In fact, if Holiday Marsh were included in the regression analyses, the r^2 values would increase substantially from 0.43 to 0.61 in Fig. 4*a* and from 0.68 to 0.81 in Fig. 4*b*, without significant alteration of the slopes (ANCOVA; $P > 0.5$ in both cases).

By comparison, Big Creek Marsh (wetland 2 in Fig. 4) is probably truly anomalous, since it had relatively good water and sediment quality, even though it is located in a heavily agricultural watershed and is also subject to carp disturbance (Table 1; Fig. 1). Inclusion of Big Creek to the regression analyses lowered the r^2 values from 0.43 to 0.30 in Fig. 4*a* and from 0.68 to 0.42 in Fig. 4*b*. One possible explanation for this departure is that the substrate in the Big Creek watershed is primarily sandy, whereas soils in other heavily farmed wetlands are dominated by clay and silt. The larger and heavier sand particles are known to settle out of the water column more rapidly than clay and silt particles, and this may explain why water turbidity in Big Creek Marsh was consistently low. Clay particles also tend to be ladened with adsorbed phosphorus, whereas sand particles do not (Golterman 1995), and this difference may account for the low phosphorus concentrations in Big Creek Marsh compared with other wetlands. In contrast, accompanying nitrate levels were extremely high in Big Creek, likely due to the sod farms in the area that use fertilizers that favour a high nitrogen ratio. Since nitrogen is generally the limiting factor for macrophyte growth (Barko et al. 1991), the combination of clear water and excess nitrate may have promoted luxuriant growth of submergent plants, despite the dominance of agricultural lands in the watershed.

Urban land use was not a significant predictor of water or sediment quality of wetlands in this study. However, urban wetlands were poorly represented in our data set, and ongoing research in our laboratory confirms that relatively few urban wetlands currently exist in southern Ontario because marshes in heavily settled areas of the province have generally been eliminated due to dredging or infilling. Thus, further attempts to increase representation of urban wetlands may not be successful.

Previous studies have shown that submergent plant cover (Robel 1962) and water clarity (Lougheed et al. 1998) can be related to the biomass of carp in wetlands. Such results have been used to justify carp exclusion/reduction as a means to restore Great Lakes coastal wetlands (e.g., Cootes Paradise Marsh and Oshawa Second Marsh). Results of this study, however, suggest that a greater emphasis on wetland conservation and restoration in the Great Lakes should be focussed on controlling non-point-source impacts because there was no consistent effect of carp resuspension on either water turbidity or the species richness of submergent vegetation. The chief determinant of water and sediment quality was the proportion of agricultural land (modified index), which accounted for about 70% of the overall variation in trophic quality. The remaining variation may be explained by such factors as carp biomass, wind and wave resuspension, and substrate type. We recommend that future studies be conducted that consider these variables simultaneously for a large number of wetlands so that the relative impacts of each can be quantified.

We have suggested that water quality impairment associated with extensive farming can be ameliorated through provision of forested buffer strips. Previous work by Peterjohn and Correll (1984) also demonstrated that riparian vegetation and forests can filter out a great deal of phosphorus, nitrogen, and suspended material in surface runoff and thus reduce loading to downstream sites. Differences in subsurface contribution of dissolved phosphorus can also be affected by the tillage practice used (Gaynor and Findlay 1995). Other best-management practices such as those oriented towards minimizing the impact of confined animal operations will also reduce the sediment and nutrient load from agricultural watersheds (Edwards et al. 1996).

The effects of buffer strips were not quantitatively evaluated in this study because it was not within the scope of this

© 1999 NRC Canada

paper to fully explore their effectiveness. Nevertheless, by applying a conservative factor to our data based on benefits observed elsewhere, we hope that our results will encourage other investigators to conduct field studies to link the management value of buffer strips to the water quality of downstream wetlands. We need to quantify the beneficial effects of different types of best-management practice throughout the Great Lakes basin to provide a scientific basis for recommending the appropriate strategy to retard further degradation of wetlands in heavily farmed catchments.

Although we grouped together agriculture of all types in this study, we acknowledge that the impacts of crop farming versus cattle farming are very different (Beaulac and Reckhow 1982). Many types of farming subject the soil to long periods of exposure with correspondingly high rates of erosion. Different application rates of various types of fertilizers and herbicides will also lead to differences in pollutant loads to wetlands via runoff. Combining the power of recent geographic information systems technology with empirically derived export coefficients for the Great Lakes basin should improve the predictive power of land use models (e.g., Mattikalli and Richards 1996) and improve our ability to predict non-point-source impacts on coastal wetlands.

There is good reason why we focussed on the submergent plant community as a primary indicator of wetland quality. Submersed macrophytes spend all or most of their life cycle inside the water column and, as such, compete with algae for light. Whereas nutrient uptake by algae is through the water, macrophytes primarily utilize nutrients from the sediment (Carignan and Kalff 1980). Therefore, nutrients in the water column will disproportionately benefit the algae and allow them to shade out the macrophytes, especially in wetlands where eutrophic conditions affect water clarity. This situation is responsible for the well-documented alternate states of shallow lakes throughout the world: turbid, algae-dominated systems devoid of macrophytes or clear, macrophyte-dominated systems (e.g., Moss 1990; Chow-Fraser 1998).

Decrease in species richness with increasing water turbidity can be explained in terms of Grime's (1979) theoretical framework, in which he proposed three main types of adaptive strategies for vascular plants depending on the degree of disturbance and stress in their habitat. Wetlands with low PC1 scores are associated with seasonal rather than continuous disturbance (i.e., water depths are reduced only in late summer) and relatively low stress levels (i.e., low water turbidity and high sediment phosphorus content). According to Grime (1979), such an environment should favour many competitive species. By comparison, wetlands with high PC1 scores are associated with high continuous disturbance (e.g., uprooting activities of carp) as well as high stress levels (low light penetration because of high water turbidity). In such habitats, there is no viable strategy, and consequently, submergent growth is almost negligible (e.g., Big Creek Marsh, Cootes Paradise Marsh prior to carp exclusion, Lougheed et al. 1998). Once disturbance is reduced (e.g., Cootes Paradise Marsh after carp exclusion, P. Chow-Fraser, unpublished data), however, a few stress-tolerant species capable of growing in low light levels may become established (e.g., *Potamogeton pectinatus*, Lehmann et al. 1997; *Ceratophyllum demersum*, *Elodea canadensis*, and *Myriophyllum verticillatum*, Toivonen and Huttunen 1995).

We have shown that the trophic quality of 22 wetlands (located primarily in the catchment areas of Lakes Ontario and Erie) was significantly affected by the percent agricultural land in the respective watersheds, independent of in-marsh stresses such as carp disturbance. The decline in water quality from marshes with good water transparency and low nutrient concentrations to turbid systems enriched with phosphorus and nitrogen was accompanied by a predictable decrease in the species richness of submergent aquatic plants. Our study thus provides direct links among land use impacts, water quality impairment, and wetland plant diversity. The apparent compensatory effect of buffer strips on the water quality of downstream wetlands should be investigated more rigorously, since they may be very important in maintaining the diversity of submergent plants in Great Lakes coastal marshes.

Acknowledgements

This study was partially funded by the the Tri-Council Eco-Research Program for Hamilton Harbour and the Natural Sciences and Engineering Research Council of Canada. Devon Cancilla of the National Laboratory for Environmental Testing, Environment Canada, provided immunoassay kits and assistance with sample processing. We thank Vanessa Lougheed for field and laboratory assistance and for providing constructive reviews of earlier drafts, along with two anonymous reviewers. We extend gratitude to the staff of Conservation Authorities and the Ontario Ministry of Natural Resources field offices, Cathy Moulder of the McMaster University map library, and the students of Biology 4Y03. Our thanks to Chris Diggin who got us airborne to make visual surveys of remote wetlands.

References

American Public Health Association. 1992. Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association, Washington, D.C.

Andersen, J.M. 1976. An ignition method for determination of total phosphorus in lake sediments. Water Res. **10**: 329–331.

Barko, J.W., Gunnison, D., and Carpenter, S.R. 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquat. Bot. **41**: 41–65.

Beaulac, M.N., and Reckhow, K.H. 1982. An examination of land use – nutrient export relationships. Water Resour. Bull. **18**: 1013–1024.

Carignan, R., and Kalff, J. 1980. Phosphorus sources for aquatic weeds: water or sediments? Science (Washington, D.C.), **207**: 987–989.

Chow-Fraser, P. 1998. A conceptual model to aid restoration of Cootes Paradise Marsh, a degraded coastal wetland of L. Ontario, Canada. Wetland Ecol. Manage. **6**: 43–57.

Chow-Fraser, P., Lougheed, V.L., Crosbie, B., Le Thiec, V., Simser, L., and Lord, J. 1998. Long-term response of the biotic community to fluctuating water levels and changes in water quality in Cootes Paradise Marsh, a degraded coastal wetland of L. Ontario. Wetland Ecol. Manage. **6**: 19–42.

Crosbie, B. 1997. An evaluation of the pollutants entering Ontario's wetlands: how land use impacts wetland health. M.Sc. thesis, Department of Biology, McMaster University, Hamilton, Ont.

© 1999 NRC Canada

Crowder, A., and Painter, D.S. 1991. Submerged macrophytes in Lake Ontario: current knowledg importance, threats to stability, and needed studies. Can. J. Fish. Aquat. Sci. **48**: 1539–1545.

Edwards, D.R., Daniel, T.C., Scott, H.D., Murdoch, J.F., Habiger, M.J., and Burks, H.M. 1996. Stream quality impacts of best management practices in a northwestern Arkansas basin. Water Resour. Bull. **32**: 499–509.

Ehrenfeld, J.G. 1983. The effects of changes in land-use on swamps of the New Jersey Pine Barrens. Biol. Conserv. **25**: 353–375.

Findlay, C.S., and Houlahan, J. 1997. Anthropogenic correlates of species richness in southeastern Ontario wetlands. Conserv. Biol. **11**: 1000–1009.

Frink, C.R. 1991. Estimating nutrient exports to estuaries. J. Environ. Qual. **20**: 717–724.

Gaynor, J.D., and Findlay, W.I. 1995. Soil and phosphorus loss from conservation and conventional tillage in corn production. J. Environ. Qual. **24**: 734–741.

Gaynor, J.D., MacTavish, D.C., and Findlay, W.I. 1995. Atrazine and Metolachlor loss in surface and subsurface runoff from three tillage treatments in corn. J. Environ. Qual. **24**: 246–256.

Golterman, H.L. 1995. The labyrinth of nutrient cycles and buffers in wetlands: results based on research in the Camargue (southern France). Hydrobiologia, **315**: 39–58.

Grime, J.P. 1979. Plant strategies and vegetation processes. John Wiley & Sons, New York.

Hach Company. 1989. Water analysis handbook. Hach Company, Loveland, Co.

Hough, R.A., Fornwall, M.D., Negele, R.J., Thompson, R.L., and Pritt, D.A. 1989. Plant community dynamics in a chain of lakes: principal factors in the decline of rooted macrophytes with eutrophication. Hydrobiologia, **173**: 199–217.

Johnes, P., Moss, B., and Phillips, G. 1996. The determination of total nitrogen and total phosphorus concentrations in freshwaters from land use, stock headage and population data: testing of a model for use in conservation and water quality management. Freshwater Biol. **36**: 451–473.

Johnson, L.B., Richards, C., Host, G.E., and. Arthur, J.W. 1997. Landscape influences on water chemistry in midwestern stream ecosystems. Freshwater Biol. **37**: 193–208.

Keddy, P. 1983. Freshwater wetlands human-induced changes: indirect effects must also be considered. Environ. Manage. **7**: 299–302.

Keddy, P., and Reznicek, A.A. 1986. Great Lakes vegetation dynamics: the role of fluctuating water levels and buried seeds. J. Gt. Lakes Res. **12**: 25–36.

Lehmann, A., Castella, E., and Lachavanne, J.-B. 1997. Morphological traits and spatial heterogeneity of aquatic plants along sediment and depth gradients, Lake Geneva, Switzerland. Aquat. Bot. **55**: 281–299.

Lougheed, V.L., Crosbie, B., and Chow-Fraser, P. 1998. Predictions on the effect of carp exclusion on water turbidity, zooplankton, and submergent macrophytes in a Great Lakes wetland. Can. J. Fish. Aquat. Sci. **55**: 1189–1197.

Maltby, L., Boxall, A.B.A., Forrow, D.M., Calow, P., and Betton, C.I. 1995. The effects of motorway runoff on freshwater ecosystems. 2: Identifying major toxicants. Environ. Toxicol. Chem. **14**: 1093–1101.

Mattikalli, N.M., and Richards, K.S. 1996. Estimation of surface water quality changes in response to land use change: application of the export coefficient model using remote sensing and geographical information system. J. Environ. Manage. **48**: 263–282.

Maynard, L., and Wilcox, D. 1996. Coastal wetlands of the Great Lakes: background paper for the State of the Lake Conference (SOLEC). Environment Canada and U.S. Environmental Protection Agency EPA 905-D-96-001c, Chicago and Toronto.

Moore, D.R.J., Keddy, P.A., Guadet, C.L., and Wisheu, I.C. 1989. Conservation of wetlands: do infertile wetlands deserve a higher priority? Biol. Conserv. **47**: 203–217.

Morgan, M.D., and Philipp, K.R. 1986. The effect of agricultural and residential development on aquatic macrophytes in the New Jersey Pine Barrens. Biol. Conserv. **35**: 143–158.

Moss, B. 1990. Engineering and biological approaches to the restoration from eutrophication of shallow lakes in which aquatic plant communities are important components. Hydrobiologia, **200/201**: 367–377.

Murphy, J., and Riley, J. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta, **27**: 31–36.

Nelson, P.N., Cotsaris, E., and Oades, J.M. 1996. Nitrogen, phosphorus, and organic carbon in streams draining two grazed catchments. J. Environ. Qual. **25**: 1221–1229.

Osborne, L.L., and Wiley, M.J. 1988. Empirical relationships between land use/cover and stream water quality in an agricultural watershed. J. Environ. Manage. **26**: 9–27.

Peterjohn, W.T., and Correll, D.L. 1984. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology, **65**: 1466–1475.

Robel, R.J. 1962. The relationship of carp to waterfowl food plants on a western marsh. Dep. Inf. Bull. 62-4. Utah State Department of Fish and Game, Salt Lake City.

Smith, P.G.R., Glooschenko, V., and Hagen, D.A. 1991. Coastal wetlands of three Canadian Great Lakes: inventory, current conservation initiatives, and patterns of variation. Can. J. Fish. Aquat. Sci. **48**: 1581–1594.

Speziale, B.J., Schreiner, S.P., Giammatteo, P.A., and Schindler, J.E. 1984. Comparison of *N,N*- dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. Can. J. Fish. Aquat. Sci. **41**: 1519–1522.

Toivonen, H., and Huttunen, P. 1995. Aquatic macrophytes and ecological gradients in 57 small lakes in southern Finland. Aquat. Bot. **51**: 197–221.

Whillans, T.H. 1982. Changes in marsh areas along the Canadian shoreline of L. Ontario. J. Gt. Lakes Res. **8**: 570–577.

Whillans, T.H. 1996. Historic and comparative perspectives on rehabilitation of marshes as habitat for fish in the Lower Great Lakes basin. Can. J. Fish. Aquat. Sci. **53**(Suppl. 1): 58–66.

Zaruk, D.M., Young, C., and Cancilla, D.A. 1995. The development of an immunoassay based screening program for environmental samples at the National Laboratory for Environmental Testing. WEF Specialty Conference Series Proceedings, Cincinnati, Ohio. Water Environment Federation, Alexandria, Va.

© 1999 NRC Canada

Empirical relationships between polychlorinated biphenyls in sediments and submerged rooted macrophytes

Claire Vanier, Dolores Planas, and Michel Sylvestre

Abstract: In this study, we use empirical relationships to model accumulation of polychlorinated biphenyls (PCBs) in submerged rooted macrophytes sampled in the upper St. Lawrence River (Canada – United States). The correlations between PCB congener concentrations in sediments, in macrophyte shoots, and in their roots were all significant ($P < 0.001$). However, the PCB congener concentrations in macrophyte shoots were better predicted by concentrations in sediments (top 5 cm) than by those in the roots of plants ($R^2 = 0.839$ and 0.704, respectively). Our results suggest that models based on the phase partitioning concept are applicable to submerged macrophytes. The lipids in shoots and the organic fraction in sediments are likely the phases involved in PCB partitioning between these two compartments. Between shoots and roots, the lipid phase also appears to be involved in the congener partitioning. However, between roots and sediments (5- to 10-cm depth interval), the correlation was weaker using organic weight standardisation of congener concentrations in sediments than using dry weight standardisation ($R^2 = 0.852$ and 0.778, respectively).

Résumé : Dans cette étude, nous avons développé un modèle de l'accumulation en biphéniles polychlorés (BPCs) chez des macrophytes submergés enracinés du fleuve Saint-Laurent (Canada – États-Unis), sur la base de relations empiriques. Les corrélations entre les concentrations des congénères de BPC dans les sédiments, les macrophytes et leurs racines étaient toutes significatives ($P < 0{,}001$). Toutefois, les sédiments (0–5 cm) offraient une meilleure prédiction des concentrations dans les macrophytes (tiges et feuilles) que les racines ($R^2 = 0{,}839$ et 0,704, respectivement). Selon nos résultats, les modèles basés sur le concept de partition entre phases seraient applicables à l'accumulation en BPC dans les macrophytes submergés. Les phases lipidique des macrophytes et organique des sédiments seraient impliquées dans la partition des congénères de BPC entre ces deux compartiments. La phase lipidique serait aussi impliquée dans la partition des congénères entre les macrophytes et leurs racines. Toutefois, entre les racines et les sédiments (5–10 cm), la corrélation était plus faible lorsque les concentrations des congénères étaient exprimées en poids organique des sédiments qu'en poids sec ($R^2 = 0{,}852$ et 0,778, respectivement).

Introduction

In most industrialised countries, the use of polychlorinated biphenyls (PCBs) has been restricted or banned since the late 1970s, resulting in a decline in PCB releases in the environment. However, in several aquatic ecosystems, the biota contamination remained relatively stable since the middle of 1980s (Borgmann and Whittle 1992; Picer and Picer 1995; Stow et al. 1995). Mobilisation of PCBs from sediments is one of the causes of the persistence of these contaminants (MacDonald et al. 1993; Larsson et al. 1998), since sediments were in the past the most important sink of hydrophobic pollutants.

Received April 2, 1998. Accepted June 24, 1999.
J14527

C. Vanier and D. Planas.[1] GEOTOP, Université du Québec à Montréal, C.P. 8888, Succ. Centre, Montréal, QC H3C 3P8, Canada.
M. Sylvestre. Institut National de Recherche Scientifique-Santé, Université du Québec, 245, boul. Hymus, Pointe-Claire, QC H9R 1G6, Canada.

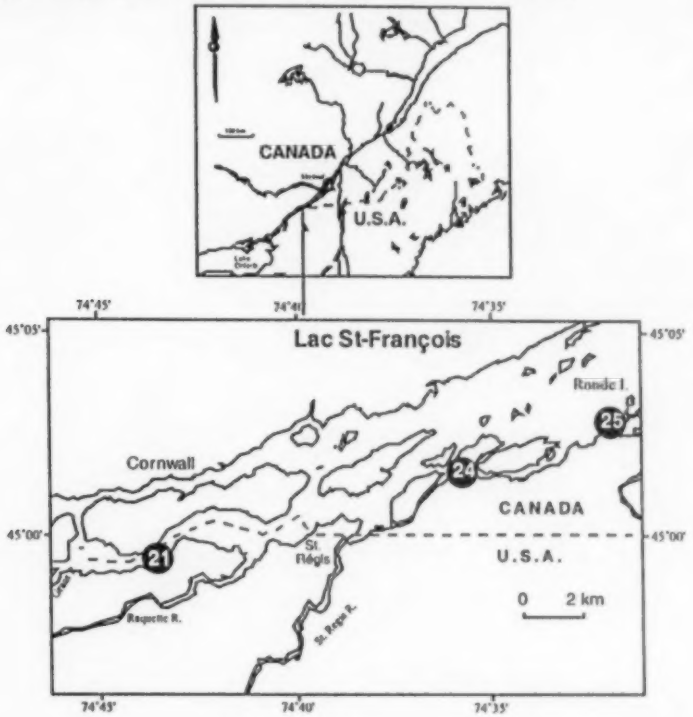
[1]Author to whom all correspondence should be addressed.
e-mail: planas.dolores@uqam.ca

Rooted macrophytes might be involved in this mobilisation and there is a growing interest in the role of these plants in the redistribution of organic contaminants in aquatic ecosystems (Stewart et al. 1992; Teil et al. 1996; Lovett-Doust et al. 1997). Rooted macrophytes have access to contaminants buried in sediments, and the concentration of contaminants in their shoots and in their roots may reach similar or higher values than in corresponding sediments (Mrozek et al. 1982; Stewart et al. 1992; Lovett-Doust et al. 1994). Experimental studies have shown that root uptake and translocation of organic contaminants between roots and shoots occur (Mrozek et al. 1982; Hinman and Klaine 1992; Lovett-Doust et al. 1994), as well as uptake in plants from water (Gobas et al. 1991; Hinman and Klaine 1992). Submerged macrophytes may reach high biomass in certain river lakes, such as those of the St. Lawrence River (Hudon 1997), and it would be useful to be able to estimate the contribution of these plants to the mobilisation of PCBs and other persistent contaminants in aquatic ecosystems. However, published field studies on PCB accumulation in macrophytes remain scarce and this limits our capacity to predict this contribution.

The bioaccumulation modelling of hydrophobic contaminants in aquatic ecosystems is often developed from the partitioning concept (Connor 1984; Gobas 1993; van der Oost

© 1999 NRC Canada

Fig. 1. Sediment and macrophyte sampling stations in Lac Saint-François (St. Lawrence River).



et al. 1996). According to this concept, the accumulation of contaminants in compartments is governed by the respective compound affinities for relevant phases (aqueous, particle, organic, or lipid phase), regardless of species or site characteristics (Mackay 1979; van der Kooij et al. 1991). Because sediments are one of the main sources of PCBs for the aquatic ecosystem and because water usually shows low concentrations and high fugacity for these compounds, empirical modelling of bioaccumulation in aquatic organisms is generally based on sediment concentrations (Mackay 1979; Gobas 1993).

Because physical–chemical characteristics of PCBs vary among congeners, modelling their environmental fate requires the correct standardisation of concentrations in each compartment. Organic weight standardisation is usually used for PCBs in sediments (Thomann et al. 1992; van der Oost et al. 1996), since organic carbon was found to increase the sorption of hydrophobic contaminants in sediments (Karickhoff et al. 1979; Mrozek et al. 1982). In the biota, the lipid fraction is considered to be the accumulation site of hydrophobic contaminants (Mackay 1979; Paterson et al. 1990; Gobas 1993), but this remains a subject of debate (Borgmann and Whittle 1992). According to Skoglund et al. (1996), models based on organic content in algae would more efficiently predict the accumulation of these contaminants than models based on lipid content. From the few data available on organochlorine accumulation and lipid content in macrophytes, correlations found between these two variables were often weak or not significant (Teil et al. 1996; Lovett-Doust et al. 1997; Richard et al. 1997).

The objective of the present study was to develop a simple empirical model to predict PCB accumulation in submerged rooted macrophytes, based on field data. To develop the model, we compared the efficiency of sediment PCBs with that of roots for predicting the congener concentrations in the shoots of the macrophytes. Predicting efficiencies were compared on the basis of the correlation (simple linear regression analyses) of PCB congener concentrations found between macrophyte shoots and sediments with that between shoots and roots. To take into account PCB congener affinities for specific phases of compartments, which could influence congener accumulation, we established and compared several relationships that differed between them by the standardisation method used to express the congener concentrations in samples (i.e., based on dry, organic, or lipid weight of samples).

Methods

Study site

The study was carried out at the end of July 1992 in Lac Saint-François, a natural enlargement of the upper St. Lawrence River (Fig. 1). The lake (surface area 233 km^2, mean depth 5 m) is the outlet of the Great Lakes and is crossed from west to east by the St. Lawrence River Seaway (depth 22 m, flow rate 2–3 $m \cdot s^{-1}$). Outside the channel, submerged macrophytes cover about 80 km^2 of the bottom area and can be found to a depth of 8.5 m. Reported dominant species are *Myriophyllum* sp., *Potamogeton* sp., *Elodea canadensis*, and *Vallisneria americana* (Armelin et al. 1994). In addition to Lake Ontario inputs, major diffuse sources of PCBs have been identified from industrial landfills on the southwestern shore of the lake, and this section of the lake is believed to be one

© 1999 NRC Canada

Table 1. PCB congeners analysed in sediments and in macrophyte shoots and roots from Lac Saint-François and their grouping in isomer classes.

Isomer class	IUPAC No.
2 CB	15 (17)
3 CB	18, 31 (28)
4 CB	40,44, 49, 52, 60
5 CB	86, 87, 101, 105, 110 (77), 114, 118 (149), 121
6 CB	128, 129, 138, 141, 151, 153 (132)
7 CB	170, 171, 173, 180, 183, 185, 187 (182)
8 CB	194, 195, 196 (203), 199
9 CB	206

Note: Coeluting congeners not quantified. CB, chlorobiphenyl.

of the most contaminated in the St. Lawrence River (Armelin et al. 1994; Vanier et al. 1996).

Sampling

The shoots of macrophytes, their roots, and adjacent sediments were sampled at three sites in Lac Saint-François (Fig. 1). Because the length of roots varies within and between macrophyte species, three sediment layers (0–5, 5–10, and 10–15 cm) were sampled to (*i*) evaluate the influence of sediment depth in the prediction of PCB congener concentrations in roots and in shoots and (*ii*) determine the best compartment (sediments or roots) for predicting PCB concentrations in shoots. Station 21 (st21) was sampled close to a contaminated landfill and the other two (st24 and st25) at 11 and 18 km downstream from st21, respectively (Fig. 1). At each station, samples were collected by SCUBA divers at five separate sampling points along the perimeter of a circle of about 60 m. Macrophyte shoots (dominant species *E. canadensis* and *Myriophyllum* sp.) in one or two 200-cm^2 quadrats were cut at their base, carried to the boat in a Nytex bag (20-μm mesh), and kept on ice in aluminum foil. Roots of macrophytes were thereafter sampled on the same quadrats, cleaned of sediments, and kept on ice in aluminum foil. Close to each quadrat, sediments were cored using metallic cylinders (30 cm long, 10 cm in diameter); the upper 15 cm of sediment was extruded and sliced at 5-cm intervals and each slice (S1 = 0–5 cm, S2 = 5–10 cm, S3 = 10–15 cm) was kept separately in a glass jar. Macrophyte shoots were cleaned of their epiphytes by stirring samples three times in a glass jar filled with filtered (0.45 μm) lake water and all samples (n = 15 for shoots, n = 14 for roots, n = 45 for sediments) were frozen (within 8 h) at –20°C until analysed. The sampling and laboratory material was washed with acetone and hexane before use.

Two 10 g wet weight aliquots of sieved sediments (2-mm mesh) were used for PCB analysis. Root and shoot samples were crushed in liquid nitrogen and two aliquots of homogenate (shoots 10 or 20 g, roots 1–3 g) were dried with sodium sulphate before PCB extraction. About 10 g of shoot and 2 g of root homogenates (wet weight) and a 10 g wet weight aliquot of sieved sediments were used for dry weight (70°C) and ash-free dry weight (500°C for 1 h) determination. The loss on ignition was assumed to represent the organic matter (OM) in samples.

PCB extraction and lipid determination

PCB extraction and quantification in sediments and in macrophyte shoots and roots were done following Vanier et al. (1996). Briefly, PCBs were extracted from each analytical duplicate of each sample by ultrasonication in hexane. The extracts were concentrated to a final volume of 4 mL (2 mL for roots) and split for PCB analysis. Before purification, sediment and root extracts were treated with activated copper in order to remove sulphur compounds. Extracts were purified with 50 mL of hexane in a column (25 × 1 cm) containing 3 g of Florisil (60/100 mesh) topped with 0.7 g of sodium sulphate. The elutes were concentrated (sediments 2 mL, roots 50 μL, shoots 100 μL) and 1 μL of the resulting concentrated extracts was used for gas chromatography (GC) analysis.

Analyses were performed on a Perkin-Elmer Sigma-2 gas chromatograph fitted with a fused silica capillary column (0.2 mm × 25 m; HP-5, phenyl–methyl–silicon) coupled to an electron capture detector (GC/ECD). The oven temperature was held at 60°C for 2 min and was programmed to increase at increments of 10°C·min^{-1} up to 130°C and then at increments of 5°C·min^{-1} up to 290°C (injector temperature 275°C, detector temperature 320°C). The quantification of individual PCB congeners (listed in Table 1) was made by comparison with CLB-1 mixture standards (National Research Council of Canada, Halifax, N.S.). Congener identification was confirmed by GC/MS (Hewlett Packard, model 5970 gas chromatograph – mass selective detector) under the same conditions as stated for the GC/ECD analysis. In the GC/MS analysis, a trichlorobiphenyl, probably PCB 17, was found to coelute with congener 15. As congener 17 was absent from the CLB-1 mixtures, the chromatographic peak was quantified on the basis of the standard for dichlorobiphenyl 15. Procedural blanks were done for every 8 to 10 samples. Congener recovery, assessed from three replicate 10-g "clean" macrophyte samples spiked with an Aroclor 1248 solution, ranged from 80 to 95% and the detection limit was between 1×10^{-4} and 5×10^{-4} mg·kg wet $weight^{-1}$, depending on the congener analysed. Hexane-extractable lipid weight was determined by evaporating the split macrophyte extracts under a nitrogen stream and weighing the residues. For roots, the lipid content was determined on pooled extracts of samples from each station because of the low biomass per sample (5 g or less).

Data analysis

The PCB concentrations in samples (analytical duplicate mean concentrations) are reported as (*i*) the sum of the concentrations of the 34 congeners quantified (total PCB concentration, milligrams per kilogram) and (*ii*) the individual concentrations (micrograms per kilogram) of these congeners. PCB concentrations were standardised on the basis of the dry (d) and organic (o) weights for all samples and of the lipid weight (li) for root and shoot samples. For these calculations, the missing values of OM (n = 39 for sediments, n = 15 for shoots, n = 11 for roots) or lipid fractions (n = 14 for shoots and for roots) in samples were replaced by the mean value found in the samples from the same station (and from the same layer, in the case of sediments). The PCBs in samples were also reported as isomer proportions, which were calculated from the sum of the relative congener concentrations grouped according to the number of chlorine atoms per molecule (see in Table 1).

Analysis of variance (one- or two-way ANOVA) or Wilcoxon/Kruskall–Wallis tests (SAS Institute, Inc. 1997) were used to compare the total PCB concentration (expressed on the basis of dry, organic, and lipid weights) and the OM or lipid contents of samples between stations, between compartments, and between layers for sediments. Relationships between the total PCB concentration and the OM or lipid content were evaluated using analysis of covariance (ANCOVA) with sampling location (i.e., station) being the factor. For roots, comparisons between stations were only done on total PCB concentrations expressed on the basis of dry and organic weights, since the lipid content of roots was determined on pooled station samples.

The relationships of the congener concentrations ($\log_{10}$) between compartments (sediments, roots, and shoots) were established by simple linear regression analysis (SAS Institute, Inc. 1997) using the different forms of standardisation for congener concentrations in samples. Concentrations were expressed on the basis of the dry and organic weights of shoots (M_d and M_o, respectively), of roots (R_d and R_o, respectively), and of the three sediment layers ($S1_d$, $S1_o$, $S2_d$, $S2_o$, $S3_d$, and $S3_o$, respectively). The lipid weight stand-

© 1999 NRC Canada

Table 2. Mean (SD) total of PCB concentrations ($mg \cdot kg^{-1}$) and % OM content in the three sediment depth intervals (S1, S2, S3) sampled at three stations on Lac Saint-François.

Station	Depth (cm)	TS_d	TS_o	% OM
21	0–5	10.67 (6.95)	157.2 (90.6)	6.54 (0.88)
	5–10	17.30 (18.13)	333.3 (384.3)	5.52 (0.69)
	10–15	29.74 (37.02)	596.6 (810.7)	5.78 (1.33)
24	0–5	1.03 (0.24)	8.5 (2.2)	12.27 (1.33)
	5–10	1.22 (0.59)	8.3 (4.6)	12.94 (0.64)
	10–15	1.39 (0.68)	10.9 (5.2)	11.92 (0.11)
25	0–5	0.51 (0.04)	5.0 (0.4)	10.04 (0.69)
	5–10	0.53 (0.06)	6.8 (3.2)	8.70 (2.55)
	10–15	0.49 (0.06)	7.2 (2.9)	7.33 (1.85)
P(*F*)	0–5	***	***	***
P(*S*)		21 > (24 ~ 25)	21 > (24 ~ 25)	24 > 25 > 21
P(*F*)	5–10	**	**	***
P(*S*)		21 > (24 ~ 25)	21 > (25 ~ 24)	24 > 25 > 21
P(*F*)	10–15	***	**	***
P(*S*)		21 > (24 ~ 25)	21 > (24 ~ 25)	24 > (25 ~ 21)

Note: Total PCB concentrations are expressed on a dry (TS_d) and organic (TS_o) weight basis. The number of replicates in each sediment layer at each station was $n = 5$ for all variables except for OM ($n = 4$) in S1 and OM ($n = 3$) in S2 and S3 at st 24 and OM ($n = 4$) in S1 at st25. Comparison between stations: *P*(*F*), ANOVA: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; *P*(*S*), Tukey–Kramer HSD: >, significantly higher ($P < 0.05$); <, significantly lower ($P < 0.05$); ~, approximately equal ($P > 0.05$).

ardisation of the concentrations was also used for macrophyte shoots (M_{li}). Relationships involving the concentrations in roots were also established at the station level, using the means of the congener concentrations of the station samples for the three compartments, so that the lipid standardisation in roots (R_{li}) could be compared with the others. The strength (R^2) of the relationships was used to determine (*i*) the best sediment layer for the prediction of the concentrations in roots and in shoots, (*ii*) the best predictor, between roots and sediments, of the congener concentrations in shoots, and (*iii*) the best standardisation of congener concentrations in each compartment.

Results

Total PCB concentrations in sediments

For each of the three sediment layers (S1, S2, S3), the total PCB concentration (TS_d, TS_o) was higher at the upstream st21, near the point source area, than at the other two stations (Table 2). These latter stations showed similar PCB concentrations (Table 2). Neither total PCB concentration nor the OM fraction differed significantly between sediment layers at any of the stations. For each layer, samples from st24 showed the highest OM content and those from st21 showed the lowest (Table 2). The OM content in the two upper sediment layers (S1, S2) was also higher at st25 than at st21 (Table 2). In an ANCOVA involving the three stations, the PCB accumulation in the top 5 cm of sediments (log $TS1_d$) was positively related ($P < 0.05$, $R^2 = 0.958$, $n = 13$) to its OM content (log OM), with slope values that differed significantly ($P < 0.0001$) between stations. With the other sediment layers, the relationships were not significant.

Total PCB concentrations in macrophytes

The total PCB concentrations in shoots (TM_d, TM_o, TM_{li}) and in roots (TR_d, TR_o) were higher at st21 than at the other two stations (Table 3). Between st24 and st25, the total PCB concentration did not differ significantly in shoots, but did in roots (Table 3). The total PCB concentration was higher in roots than in shoots, in dry as well as in organic weight standardisation (two-way ANOVA with sampling location as cofactor; for macrophyte compartments: $P < 0.0001$, $n = 29$). The OM content did not differ significantly between roots and shoots or among stations (two-way ANOVA; $P > 0.2$, $n = 26$), and lipid concentration was similar or slightly higher in roots than in shoots (Table 3). TR_d did not differ from $TS1_d$, $TS2_d$, or $TS3_d$ (two-way ANOVA with sampling location as cofactor; for compartments: $P > 0.2$, $n = 29$).

The lipid concentrations in shoots (wet weight, Li_w) did not vary significantly among stations (Table 3). However, the total PCB concentration (log TM_d) was positively related to log Li_w (ANCOVA; $P < 0.0001$, $R^2 = 0.954$, $n = 14$), and the slope value (0.336 ± 0.190, 95% confidence limits; $P < 0.01$) did not differ significantly among stations. The relationship between PCB and lipid concentrations in shoots was also significant when total PCB concentrations were standardised on the basis of organic weight (TM_o). In shoots, as in roots, there was no significant relationship between total PCB concentrations (TM_d, TR_d) and their respective OM contents.

The total PCB concentration in roots (log TR_d as log TR_o) was, however, negatively related to the OM content (log OM) of the S2 sediment layer (in both ANCOVA; $P < 0.0001$, $R^2 = 0.996$, $n = 12$). In both analyses, the slope and the intercept values differed significantly between stations ($P < 0.01$). The OM content of the S1 and the S3 sediment layers had a weak or nonsignificant effect on total PCB concentration in roots. In shoots, total PCB concentration was not related to the OM content of any of the sediment layers or of roots.

© 1999 NRC Canada

Table 3. Mean (SD) of total PCB concentrations ($mg \cdot kg^{-1}$), lipid concentrations ($mg \cdot g$ wet $weight^{-1}$, Li_w), and % OM content in macrophyte shoots and roots sampled at three stations on Lac Saint-François.

	Shoots					Roots				
Station	Tm_d	Tm_o	Tm_{li}	Li_w	% OM	Tr_d	Tr_o	Tr_{li}	Li_w	% OM
21	0.77 (0.15)	0.98 (0.21)	324.6 (53.9)	0.19 (0.09)	78.8 (3.0)	12.86 (4.63)	17.42 (6.49)	3435.8 (1402)	0.25 (-)	74.2 (6.1)
24	0.17 (0.05)	0.21 (0.07)	38.2 (34.7)	0.41 (0.20)	78.7 (1.0)	1.19 (0.47)	1.52 (0.66)	173.4 (82.2)	0.50 (-)	79.3 (4.2)
25	0.13 (0.06)	0.17 (0.07)	40.8 (22.5)	0.42 (0.51)	79.5 (2.7)	0.36 (0.05)	0.45 (0.06)	61.9 (4.5)	0.46 (-)	80.1 (2.4)
P	***	***	***	ns	ns	***	***	—	—	ns
P(S)	21 > (24 ~ 25)	21 > (24 ~ 25)	21 > (24 ~ 25)			21 > 24 > 25	21 > 24 > 25			

Note: Total PCB concentrations in shoots and in roots are expressed on a dry (TM_d and TR_d), organic (TM_o and TR_o), and lipid (TM_{li} and TR_{li}) weight basis. The number of replicates at each station was $n = 5$ for all variables except for Li_w ($n = 4$) for shoots at st25 and total PCB concentration ($n = 4$) and OM ($n = 3$) at st21 and OM ($n = 4$) at st24 and st25 for roots. Comparison between stations: *P*, ANOVA or Wilcoxon/Kruskall–Wallis test: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ns (not significant): $P > 0.05$; *P*(S), Tukey–Kramer HSD: >, significantly higher ($P < 0.05$); <, significantly lower ($P < 0.05$); ~, approximately equal ($P > 0.05$).

PCB congener relationships between compartments

Of quantified PCBs, lower chlorinated congeners (particularly congeners 15 (17), classified as 2-chlorobiphenyls) dominated in all samples, generally followed by tetra- and penta-chlorobiphenyls (Table 4). However, dominance of dichlorinated PCBs was more evident in roots and in sediments from st21 than in those from the two other stations. In shoots, the PCB composition appears to be more homogeneous among stations than in root or in sediment samples (Table 4). At each station, the PCB isomer composition was similar among the sediment layers (Table 4).

In the PCB relationships between compartments, all possible combinations of the congener concentrations (log_{10}) between sediments (S1, S2, S3), macrophyte shoots, and their roots were highly significant ($P < 0.001$), regardless of the standardisation method used (dry, organic, or lipid weight).

The dry weight standardisation of congener concentrations in sediments gave the best prediction for PCB accumulation in roots, regardless of the sediment layer used. Of the three layers, the second ($S2_d$, 5–10 cm) was the best predictor of concentrations in roots, with R_d (Fig. 2*a*) as well as with R_o ($R^2 = 0.852$ and 0.850, respectively). With concentrations in S1 or in S3, the strength of the relationship was weaker (e.g., R_d with $S1_d$, $R^2 = 0.790$, or with $S3_d$, $R^2 = 0.803$). Compared with the dry weight standardisation of concentrations in sediments, the organic weight standardisation decreased the strength of the relationships (e.g., R_d with $S1_o$, $R^2 = 0.794$, with $S2_o$, $R^2 = 0.778$, or with $S3_o$, $R^2 = 0.765$). Because lipid determination in roots was only done with pooled samples, relationships involving root data were also established at the station level. In these analyses, the correlation was slightly stronger between $S2_d$ and R_{li} ($R^2 = 0.923$) (Fig. 2*b*) than between $S2_d$ and R_d or R_o ($R^2 = 0.912$ and 0.913, respectively).

The degree of association of the congener concentrations was lower between shoot and root samples ($R^2 = 0.680$–0.704) than between roots and sediments. The R_d standardisation gave R^2 values that were slightly higher than R_o gave, the strongest of these correlations being between R_d and M_d ($R^2 = 0.704$) (Fig. 3*a*). At the station level, the lipid weight standardisation in both roots (R_{li}) and shoots (M_{li}) showed the strongest relationship ($R^2 = 0.837$) (Fig. 3*b*).

Congener concentrations in shoot samples appear to be better described by the concentrations in sediments than by those in roots, regardless of the standardisation method used. The best model was between M_{li} and $S1_o$ ($R^2 = 0.839$) (Fig.4), but $S1_d$ was also a good predictor of M_{li} ($R^2 = 0.814$). The congener concentrations expressed on dry or organic weight of shoots (M_d, M_o) were better predicted by $S2_d$ ($R^2 = 0.782$ in both cases). In analyses applied at the station level, sediment ($S1_o$) remained the best predictor of congener concentrations in shoots (M_{li}, $R^2 = 0.865$).

Discussion

PCB relationships between compartments

The significant relationships that we found between congener concentrations in sediments and roots and those between roots and shoots suggest a transfer of PCBs from sediments (S2) to roots and from roots to macrophyte shoots. The alternative pathway, i.e., the uptake of PCBs

© 1999 NRC Canada

Table 4. Mean (SD) of PCB isomer percentage in macrophyte shoots and roots and in the three layers of sediments (S1, S2, S3) sampled at three stations on Lac Saint-François.

Isomer class	Station	Shoots	Roots	S1 (0–5 cm)	S2 (5–10 cm)	S3 (10–15 cm)
2 CB	21	43.68 (4.30)	62.75 (7.67)	56.98 (6.06)	62.92 (10.84)	63.28 (9.91)
3 CB		13.67 (1.76)	10.07 (1.29)	11.10 (1.54)	9.44 (3.04)	10.12 (2.49)
4 CB		18.49 (2.38)	13.94 (1.93)	15.34 (1.94)	13.93 (3.61)	14.15 (2.60)
5 CB		17.73 (2.66)	8.61 (3.57)	11.23 (1.88)	8.82 (3.27)	7.85 (4.06)
6 CB		3.93 (0.72)	2.25 (1.30)	2.56 (0.51)	2.13 (0.68)	1.90 (0.88)
7 CB		1.60 (0.17)	1.47 (1.04)	1.59 (0.20)	1.51 (0.40)	1.44 (0.41)
8 CB		0.83 (0.08)	0.92 (0.51)	1.08 (0.10)	1.16 (0.42)	1.14 (0.22)
9 CB		0.34 (0.23)	0.04 (0.04)	0.14 (0.05)	0.14 (0.09)	0.14 (0.10)
2 CB	24	40.13 (6.39)	20.31 (4.75)	30.53 (4.23)	27.18 (6.14)	26.37 (4.52)
3 CB		14.22 (1.93)	11.29 (2.19)	12.38 (0.44)	12.26 (0.51)	11.92 (0.46)
4 CB		21.27 (3.28)	27.82 (1.69)	22.17 (1.28)	23.47 (1.84)	23.01 (0.93)
5 CB		16.91 (2.46)	27.27 (3.05)	22.57 (1.15)	24.36 (1.66)	25.07 (1.81)
6 CB		5.20 (1.14)	8.00 (1.74)	6.64 (0.40)	7.44 (1.12)	7.45 (0.38)
7 CB		2.15 (0.40)	3.38 (0.72)	3.53 (0.42)	3.75 (0.91)	3.85 (0.61)
8 CB		0.82 (0.47)	1.85 (0.76)	2.09 (0.71)	1.69 (0.52)	2.12 (0.44)
9 CB		0.14 (0.31)	0.31 (0.22)	0.44 (0.41)	0.15 (0.34)	0.33 (0.32)
2 CB	25	35.28 (5.44)	37.11 (8.45)	31.81 (2.86)	33.19 (5.92)	33.55 (7.41)
3 CB		13.06 (1.86)	9.31 (1.15)	10.60 (0.42)	10.66 (0.96)	11.08 (1.71)
4 CB		21.21 (1.89)	24.10 (6.03)	20.58 (1.27)	21.57 (1.66)	22.88 (3.92)
5 CB		20.13 (2.73)	20.00 (2.08)	21.46 (1.70)	21.64 (2.58)	20.66 (2.88)
6 CB		6.64 (1.50)	5.85 (1.34)	7.61 (0.87)	6.82 (0.59)	6.27 (0.98)
7 CB		2.98 (0.23)	3.50 (1.53)	5.00 (0.67)	3.77 (0.39)	3.52 (1.06)
8 CB		1.04 (0.46)	1.39 (1.28)	2.96 (0.37)	2.14 (0.22)	2.02 (0.44)
9 CB		0.00 (0.00)	0.00 (0.00)	0.82 (0.18)	0.55 (0.46)	0.29 (0.27)

Note: Congeners grouped in the isomer classes are identified in Table 1; 2 CB represents congeners 15 (17) (see Methods). The number of replicates was $n = 5$ at each station except for st21 ($n = 4$).

from the open water by shoots and their transfer to roots, seems unlikely, since PCB concentrations were much higher in roots than in shoots. The higher PCB concentrations in roots than in shoots cannot be imputed to the slight higher lipid content in roots (Table 3). Therefore, the difference in the PCB concentrations among plant parts suggests that, if there has been transfer of PCBs from the roots to the shoots, the transfer was not complete. Transfer resistance or differences in the metabolic rates of PCBs between roots and shoots (Mrozek et al. 1982) might have contributed to the differences in the concentration (Table 3) and in the composition (Table 4) of PCBs between our shoot and root samples. In a review of the uptake of organic chemical by plants, Paterson et al. (1990) noted that the transfer from roots to shoots was often greater for organic chemicals of intermediate solubility. In fact, the PCB accumulation in shoots might also have taken place independently of accumulation in roots.

Indeed, the fact that surface sediment PCB congener concentrations ($S1_o$) better predicted congener concentrations in macrophyte shoots than did root concentrations suggests an alternative pathway for PCB accumulation in the shoots. This pathway might have been an uptake by shoots of the PCBs released into open water from surface sediments, both release and uptake being governed by the specific congener affinities for the relevant phases in compartments. It is likely that with the elimination of industrial PCB usage, sediments are the main source of PCBs for organisms in Lac Saint-François, as in several other aquatic ecosystems (MacDonald et al. 1993; Larsson et al. 1998). If the atmosphere or the water had been the main source of PCBs for the lake, we should expect similar PCBs concentrations in macrophytes independently of sampled sites, which was not the case in the present study (see in Table 3).

Field studies on PCB accumulation in macrophytes and in their roots or in sediments are scarce, and this limits our capacity to compare our results with the literature. Lovett-Doust et al. (1994) studied the PCB contamination in *V. americana* during three periods of the growing season in the St. Clair and Detroit rivers (United States). When we applied our modelling approach to these data, the correlations that we found between congener concentrations in leaves and in roots of plants were weak at both sites and for all sampling periods. The strongest relationships were for the October samples taken from the most contaminated site (St. Clair River) ($P < 0.01$, $R^2 \approx 0.700$, for both dry and lipid weight standardisation of congener concentrations in samples). We also tested our model using data from Teil et al. (1996), who estimated the PCB congener concentration and composition in three macrophyte species (*Myriophyllum verticillatum*, *Ceratophyllum submersum* L., *Ranunculus fluitans*) and in sediments and water ("raw" water) at two sites along the Seine River (France). According to the authors, the congener composition in their macrophyte samples was similar to that in water. However, when we established the relationships between congener concentrations in macrophytes and those in sediments or in water, only sediment data led to significant but weak correlations. The strongest

© 1999 NRC Canada

Fig. 2. Relationships of PCB congener concentrations in the 5- to 10-cm sediment layer (dry weight) with those in roots (*a*) for all samples (dry weight in roots) and (*b*) at the station level (lipid weight in roots).

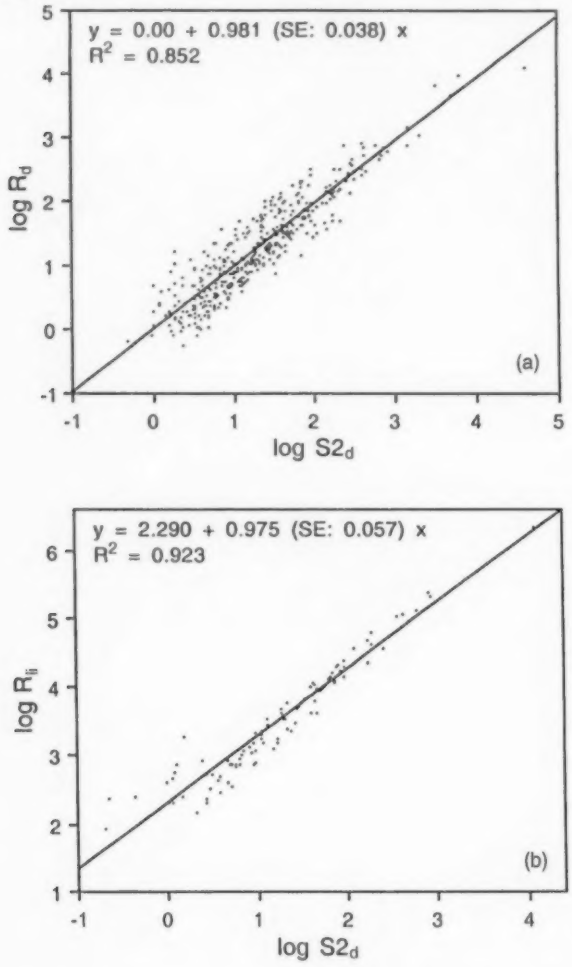
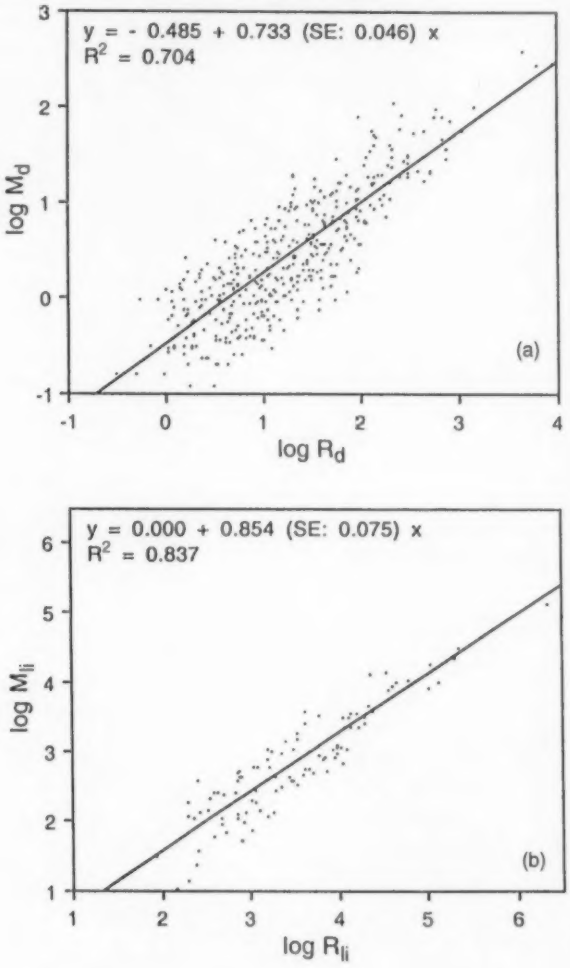


Fig. 3. Relationships of PCB congener concentrations in shoots with those in roots (*a*) for all samples (both in dry weight) and (*b*) at the station level (both in lipid weight).



sediment to shoot relationship ($P < 0.01$, $R^2 = 0.492$) was found with the *M. verticillatum* samples from the less contaminated site (Marny).

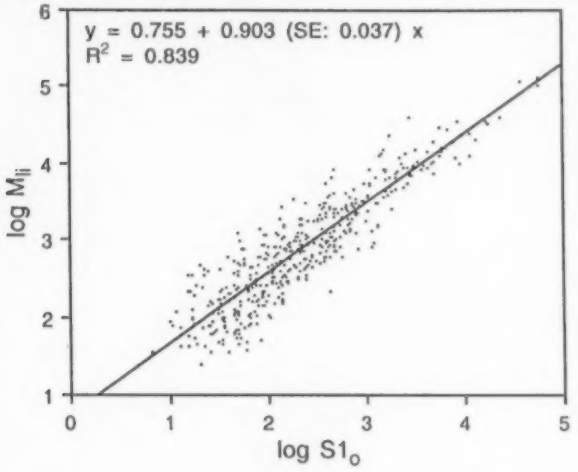
The limited available data on the PCB contamination in aquatic compartments that might be involved in the PCB accumulation in submerged macrophytes did not allow us to further test our model. In the present study, the fact that PCB accumulation in macrophyte shoots was better predicted by concentrations in sediments than by those in roots does not mean that there is no transfer between roots and shoots; rather, these findings suggest that the model based on the partitioning of PCB congeners between sediments and shoots more accurately predicts the accumulation in our macrophyte shoot samples than the models based on root data. It might indicate that there is no need to take into account the root–shoot translocation to explain the PCB accumulation in macrophyte shoots, but this needs to be confirmed with more extensive field studies.

PCB standardisation

In our samples, the lipid fraction seems to be the most important phase involved in PCB accumulation in shoots as well as in roots of macrophytes. Indeed, in all the relationships, the lipid weight standardisation of congener concentrations in both roots and shoots (R_{li}, M_{li}) yielded the strongest correlations among compartments. The significant correlation found between total PCB concentration (log TM_d) and lipid content (log Li_w) of macrophyte shoots also suggests that lipids are the preferred PCB accumulation sites in these plant tissues. This is in agreement with several authors who considered that the most determinant factor in the accumulation of hydrophobic contaminants in organisms is their lipid content (Mackay 1979; van der Kooij et al. 1991; Gobas 1993). However, Richard et al. (1997) did not find any significant relationship between PCBs and lipid concentrations in macrophytes sampled in Lac Saint-François. But in this latter study, we do not know if the statistical analyses

© 1999 NRC Canada

Fig. 4. Relationship between PCB congener concentrations in all samples of macrophyte shoots (lipid weight) and in the 0- to 5-cm sediment layer (organic weight).



applied to the data had taken into account the difference in PCB concentrations in samples between the two shores of the lake (such as in ANCOVA). Lovett-Doust et al. (1997) also did not find a significant relationship between lipid content and PCB concentrations in drifting macrophytes from the Detroit River collected in a water-intake screen at different times throughout the year. This lack of relationship might be due to the state of decomposition of the plants, given the faster degradation of lipids compared with that of PCBs (Larsson et al. 1998).

In sediments, the organic fraction is considered to be the phase that most efficiently retains hydrophobic contaminants (Karickhoff et al. 1979). This seems to have been the case in the present study, at least for the top sediment layer (S1), where the total PCB concentration was positively and significantly related to the OM content. Furthermore, congener concentrations standardised on the basis of the organic weight of these sediments ($S1_o$) gave the best prediction of PCB accumulation in shoots (M_{li}). This suggests that the affinity of congeners for the lipids in shoots and for the organic phase of sediments would control accumulation in the macrophyte shoots. In deeper sediments, the faster diagenesis of OM, relative to the persistence of PCBs, might have contributed to the lack of a significant relationship between OM and PCB concentrations. This might also explain why using the organic weight standardisation of congener concentrations in sediments ($S2_o$) decreased the predictive power (R^2 value) of the relationship between sediments and roots, relative to dry weight standardisation ($S2_d$).

Total PCB burden in macrophytes

About 80 km^2 of the bottom surface of Lac Saint-François is covered by submerged macrophytes (Armelin et al. 1994). Assuming that the mean density of macrophytes at our stations (0.084 ± 0.046 kg dry weight·m^{-2}, n = 15) and the PCB concentration found in macrophyte shoots of the less contaminated stations (st24 and st25: mean TM_d = 0.15 ± 0.05 mg·kg^{-1}, n = 10) were representative of, respectively, the density and the PCB concentrations in submerged macrophytes of the lake in the midsummer of 1992, the total PCB burden in these aquatic plants would be 1.01 kg in this period. As the sum of the 34 congeners quantified makes up 67% of the Aroclor 1248 mixture, submerged macrophytes in Lac Saint-François would have accumulated at least 1.5 kg of PCBs, in Aroclor 1248 equivalents, in the midsummer of 1992. The use of the Richard et al. (1997) data for the PCB concentrations in *M. spicatum* sampled from June to August in Lac Saint-François (mean PCB concentration = 0.085 mg·kg dry weight^{-1}, n = 5) leads to a similar but lower estimate of the total PCB burden in macrophytes (0.85 kg Aroclor 1248 equivalent value). However, both assessments probably underestimate the maximum PCB burden in submerged macrophytes of the lake, as well as their total contribution to the mobilisation of PCBs. Maximum PCB burden is probably reached later in the summer, since macrophyte biomass and PCB concentration both increase over the growing season (Mrozek et al. 1982; Lovett-Doust et al. 1994). Furthermore, our estimates did not take into account the constant drift of macrophyte fragments (turnover of leaves). In a temporal study carried out in our laboratory (M. Desrosiers, personal communication), the biomass of submerged macrophytes, sampled downstream in the St. Lawrence River, was found to increase significantly between July and August (0.24 ± 0.12 and 1.68 ± 0.85 kg dry weight·m^{-2}, respectively). The biomass slightly decreased in September but still remained close to three times that of July (0.71 ± 0.15 kg dry weight·m^{-2}). The application of these results to our data leads to a PCB burden estimated at 4.5 kg (in Aroclor 1248 equivalents) in submerged macrophytes of Lac Saint-François in September. From these assessments, it follows that about 4.5 kg of PCBs would have been released into the lake, at the macrophyte senescence. These calculations, even if they remain approximations, illustrate the potential of submerged macrophytes to accumulate and mobilise sediment-bound PCBs and other hydrophobic contaminants in aquatic ecosystems.

Acknowledgements

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada, Saint Lawrence Center, FODAR grants (University of Quebec) to D.P. and M.S., and Natural Sciences and Engineering Research Council of Canada and Eco-Research scholarships to C.V. We thank the Mohawk Council of Akwesasne, the Environment Division of St. Regis, the Tribal Council, Environment Division, and particularly K. Jock, H. Likers, and J. Ranson for the use of their facilities, M.-F. Lajoie, I. Guillemette, and M. Haviernick for their assistance in the laboratory, and Environment Canada (Saint Lawrence Center) for their logistic support.

References

Armelin, A., Mousseau, P., and Turgeon, P. 1994. Synthèse des connaissances sur les communautés biologiques du Lac Saint-François. Rapp. Tech. ZIP 1 et 2. Environnement Canada, Region du Québec, CSL, Montréal, Qué.

Borgmann, U., and Whittle, D.M. 1992. DDE, PCB, and mercury concentration trends in Lake Ontario rainbow smelt (*Osmerus*

© 1999 NRC Canada

mordax) and slimy sculpin (*Cottus cognatus*):1977 to 1988. J. Gt. Lakes Res. **18**: 298–308.

Connor, M.S. 1984. Fish/sediment concentration ratios for organic compounds. Environ. Sci. Technol. **18**: 31–35.

Gobas, F.A.P.C. 1993. A model for predicting the bioaccumulation of hydrophobic organic chemicals in aquatic food-webs: application to Lake Ontario. Ecol. Model. **69**: 1–17.

Gobas, F.A.P.C., McNeil, E.J., Lovett-Doust, L., and Haffner, G.D. 1991. Bioconcentration of chlorinated aromatic hydrocarbons in aquatic macrophytes. Environ. Sci. Technol. **25**: 924–929.

Hinman, M.L., and Klaine, S.J. 1992. Uptake and translocation of selected organic pesticides by rooted aquatic plant *Hydrilla verticillata* Royle. Environ. Sci. Technol. **26**: 609–613.

Hudon, C. 1997. Impact of water level fluctuations on St. Lawrence River aquatic vegetation. Can. J. Fish. Aquat. Sci. **54**: 2853–2865.

Karickhoff, S.W., Brown, D.S., and Scott, T.A. 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res. **13**: 241–248.

Larsson, P., Lennart, O., and Cronberg, G. 1998. Turnover of polychlorinated biphenyls in an oligotrophic and an eutrophic lake in relation to internal lake processes and atmospheric fallout. Can. J. Fish. Aquat. Sci. **55**: 1926–1937.

Lovett-Doust, J., Lovett-Doust, L., Biernacki, M., and Lazar, R. 1997. Organic contaminants in submerged macrophytes drifting in the Detroit River. Can. J. Fish. Aquat. Sci. **54**: 2417–2427.

Lovett-Doust, L., Lovett-Doust, J., and Biernacki, M. 1994. American wildcelery, *Vallisneria americana*, as a biomonitor of organic contaminants in aquatic ecosystems. J. Gt. Lakes. Res. **20**: 333–354.

MacDonald, C.R., Metcalfe, C.D., Balch, G.C., and Metcalfe, T.L. 1993. Distribution of PCB congeners in seven lake systems: interactions between sediments and food-web transport. Environ. Toxicol. Chem. **12**: 195–224.

Mackay, D. 1979. Finding fugacity feasible. Environ. Sci. Technol. **13**: 1218–1223.

Mrozek, E., Jr, Seneca, E.D., and Hóbbs, L.L. 1982. Polychlorinated biphenyl uptake and translocation by *Spartina alterniflora* Loisel. Water Air Soil Pollut. **17**: 3–15.

Paterson, S., Mackay, D., Tam, D., and Shiu, W.Y. 1990. Uptake of organic chemical by plants: a review of processes, correlations and models. Chemosphere, **21**: 297–331.

Picer, M., and Picer, N. 1995. Levels and long-term trends of polychlorinated biphenyls and DDT's in mussels collected from the eastern Adriatic coastal water. Water Res. **29**: 2707–2719.

Richard, M.R., Fox, M., and Pick, F.R. 1997. PCB concentrations and congener composition in macrophytes and sediments in the St. Lawrence River near Cornwall, Ontario. J. Gt. Lakes Res. **23**: 297–306.

SAS Institute, Inc. 1997. JMP IN, version 3.2.1. SAS Institute, Inc., Cary, N.C.

Skoglund, R.S., Stange, K., and Swackhamer, D.L. 1996. A kinetics model for predicting the accumulation of PCBs in phytoplankton. Environ. Sci. Technol. **30**: 2113–2120.

Stewart, A.J., Haynes, G.J., and Martinez, M.I. 1992. Fate and biological effects of contaminated vegetation in a Tennessee stream. Environ. Toxicol. Chem. **11**: 653–664.

Stow, C.A., Carpenter, S.R., Eby, L.A., Amrhein, J.F., and Hesselberg, R.J. 1995. Evidence that PCBs are approaching stable concentrations in Lake Michigan fishes. Ecol. Appl. **5**: 248–260.

Teil, M.J., Blanchard, M., Carru, A.M., Chesterikoff, A., and Chevreuil, M. 1996. Partition of metallic and organochlorinated pollutants and monoorthosubstituted PCB pattern in the trophic web from different areas of the River Seine. Sci. Total Environ. **181**: 111–123.

Thomann, R.V., Connolly, J.P., and Parkerton, T.F. 1992. An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. Environ. Toxicol. Chem. **11**: 615–629.

van der Kooij, L.A., van de Meent, D., van Leeuwen, C.J., and Bruggeman, W.A. 1991. Deriving quality criteria for water and sediment from aquatic toxicity tests and products standard: application of the equilibrium partitioning method. Water Res. **25**: 697–705.

van der Oost, R., Opperhuizen, A., Satumalay, K., Heida, H., and Vermeulen, N.P.E. 1996. Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*). 1. Bioaccumulation: biota–sediment ratios of PCBs, OCPs, PCDDs and PCDFs. Aquat. Toxicol. **35**: 21–46.

Vanier, C., Sylvestre, M., and Planas, D. 1996. Persistence and fate of PCBs in sediments of the Saint Lawrence River. Sci. Total Environ. **192**: 229–244.

© 1999 NRC Canada

A simple and precise method for measuring ammonium in marine and freshwater ecosystems

Robert M. Holmes, Alain Aminot, Roger Kérouel, Bethanie A. Hooker, and Bruce J. Peterson

Abstract: The accurate measurement of ammonium concentrations is fundamental to understanding nitrogen biogeochemistry in aquatic ecosystems. Unfortunately, the commonly used indophenol blue method often yields inconsistent results, particularly when ammonium concentrations are low. Here, we present a fluorometric method that gives precise measurements of ammonium over a wide range of concentrations and salinities emphasizing submicromolar levels. The procedure not only solves analytical problems but also substantially simplifies sample collection and preservation. It uses a single working reagent (consisting of orthophthaldialdehyde, sodium sulfite, and sodium borate) that is stable for months when stored in the dark. The working reagent and sample can be mixed immediately after sample collection and the reaction proceeds to completion within 3 h at room temperature. Matrix effects and background fluorescence can be corrected without introducing substantial error. This simple method produces highly reproducible results even at very low ammonium concentrations.

Résumé : La mesure exacte des concentrations d'ammonium s'avère essentielle à la connaissance de la biochimie de l'azote dans les écosystèmes aquatiques. Malheureusement, la méthode au bleu d'indophénol généralement utilisée donne souvent des résultats incohérents, notamment lorsque les concentrations d'ammonium sont faibles. Nous présentons ici une méthode fluorométrique qui permet de déterminer avec exactitude une large gamme de concentrations d'ammonium et de salinités notamment au niveau sub-micromolaire. En plus de résoudre des problèmes analytiques, cette procédure simplifie de façon appréciable la collecte et la conservation des échantillons. Elle fait appel à un seul réactif (constitué d'orthophtaldiadéhyde « OPA », de sulfite de sodium et de borate de sodium) qui demeure stable pendant des mois lorsque conservé à l'obscurité. Le réactif et l'échantillon peuvent être mélangés immédiatement après le prélèvement et la réaction s'effectue en trois heures à la température ambiante. Les écarts dus aux effets de matrice et à la fluorescence de fond peuvent être corrigés sans introduire une erreur appréciable. Cette méthode simple donne des résultats très faciles à reproduire même à de très faibles concentrations d'ammonium.

[Traduit par la Rédaction]

Introduction

Ammonium determination is one of the most common yet troublesome measurements made by limnologists and oceanographers. A recent intercomparison study involving more than 100 laboratories worldwide demonstrated that the marine community as a whole cannot measure ammonium concentrations accurately (Aminot et al. 1997), particularly at submicromolar concentrations, which are the norm in many systems (Fig. 1). It is unlikely that the freshwater community can do any better. At a minimum, uncertainty in ammonium measurements hampers our ability to discern the details of nitrogen cycling in aquatic ecosystems; at worst, it leads to incorrect conclusions about ecosystem processes or invalid comparisons across sites. For example, errors in measurement of ammonium concentration are propagated throughout complex models of nitrogen cycling, making invalid subsequent inferences.

A number of methods exist for measuring ammonium (Garside et al. 1978; Goyal et al. 1988; Genfa and Dasgupta 1989; Gibb et al. 1995; Kérouel and Aminot 1997), with the indophenol blue technique being by far the most widely used (Solorzano 1969; Brzezinski 1987; Catalano 1987). The continued publication of methods attests to the general dissatisfaction with existing techniques. All of these methods have some merit, but most suffer from lack of generality or overcomplexity. Specific criticisms of the indophenol method include the presence of high and variable blanks and difficulties with low concentration samples (Patton and Crouch 1977; Catalano 1987). In addition, the reagents are toxic and must be disposed of as hazardous waste.

The method that we present here is an adaptation of a recently published continuous-flow fluorometric technique (Kérouel and Aminot 1997). Our modified method optimizes analysis of samples with low ammonium concentrations (<0.5 µmol·L^{-1}), but it is also applicable to samples with high concentrations. It is a manual method requiring no specialized equipment other than a fluorometer, in contrast with other fluorometric methods requiring high-performance liquid chromatography separations and continuous-flow instru-

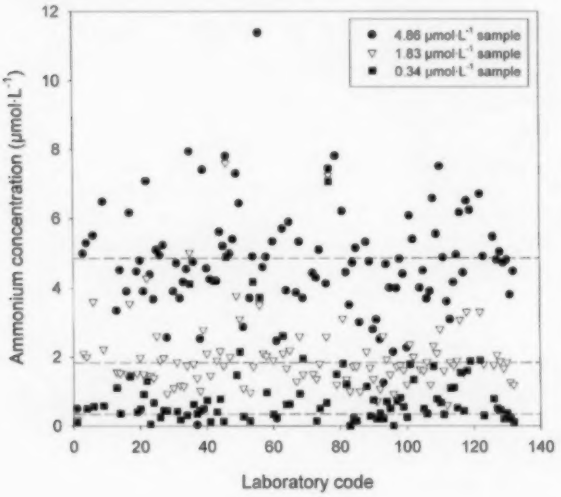
Received March 9, 1999. Accepted July 10, 1999.
J15053

R.M. Holmes,[1] B.A. Hooker, and B.J. Peterson. The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA.
A. Aminot and R. Kérouel. IFREMER-Brest, BP 70, 29280 Plouzané, France.

[1]Author to whom all correspondence should be addressed. e-mail: rholmes@mbl.edu

© 1999 NRC Canada

Fig. 1. Results of International Council for the Exploration of the Sea intercomparison study for ammonium in seawater. The horizontal broken lines show the "true" concentrations of the three samples that were sent to each of 132 participating laboratories. Modified from Aminot et al. (1997).



mentation (Gardner and St. John 1991; Kérouel and Aminot 1997).

We begin by discussing general considerations about measuring ammonium, next describe the reagents and equipment needed for the manual fluorometric method, and then present the step-by-step procedure. We then show how the method was validated using laboratory standards and consider how to deal with background fluorescence (sample autofluorescence) and matrix effects. Finally, we apply the method to various marine and freshwater samples. We conclude that the method is broadly applicable, relatively simple, and accurate even at very low ammonium concentrations.

Materials and methods

We present two variations of the method. The first (Protocol A) is optimized for samples with submicromolar ammonium concentrations. It uses large-volume samples and a high sample to reagent ratio. The second variant (Protocol B) is well suited for samples with higher ammonium concentrations. It uses a smaller sample volume and a lower sample to reagent ratio, which minimizes matrix effects but decreases sensitivity. We emphasize Protocol A, but we show how Protocol B may be preferable when ammonium concentrations generally exceed ~0.5 µmol·L^{-1}.

General considerations for measuring ammonium

A successful method for measuring ammonium in natural waters should address (*i*) sample collection in the field, (*ii*) sample preservation between collection and analysis, and (*iii*) analysis of the sample in the laboratory ("laboratory" is used generically and includes shipboard or even streamside). Too often, insufficient attention is given to sample collection and preservation; perfect measurements in the laboratory are meaningless if the sample's ammonium concentration does not reflect precisely the ammonium concentration at the field site when it was collected.

Because sources of contamination during sample collection and preservation are numerous and varied, great care must be taken if contamination is to be avoided using this or any other method. Part of the problem is that sample collection and preservation procedures vary greatly among investigators. Samples may be filtered during collection, upon returning to the laboratory, or not at all. Furthermore, samples may be analyzed immediately upon returning to the laboratory or "preserved" until later analysis by refrigerating, freezing, acidifying, or adding toxic compounds such as mercuric chloride or chloroform. These various techniques may impact the ammonium concentration of the sample differently and make comparison across sites tenuous.

Some steps that we use to minimize contamination and insure that the sample's concentration remains unchanged between collection and analysis are to (*i*) use large-volume samples (typically 80 mL for Protocol A but only 2.5 mL for Protocol B), (*ii*) use sample bottles with solid plastic caps (no liners), (*iii*) add the reagent when the sample is collected, and (*iv*) not open the sample bottle until just prior to reading on the fluorometer. We prefer relatively large-volume samples because contamination effects may be relatively smaller. In addition, larger sample bottles with wide mouths (we use 125-mL Pyrex media bottles for low-ammonium samples) are easier to clean, as are caps without liners. The reagent is added in the field immediately after sample collection, eliminating the need for sample preservation. Samples are always kept capped and are not transferred from their original bottles until just prior to fluorometric analysis, thus eliminating contamination from atmospheric ammonia. Finally, because the process of filtration can be problematic (Eaton and Grant 1979; Schierup and Riemann 1979) and since fluorescence is relatively unaffected by particulates, samples are generally not filtered unless the water is highly turbid.

In some cases, it may be impractical to begin incubations immediately after sample collection. In these cases, samples should be preserved as well as possible and returned to the laboratory for later reagent addition and analysis, recognizing that adequate preservation may be impossible for samples with very low ammonium concentrations. If sample preservation is unavoidable, we suggest filtration in the field, ideally using an in-line filtration system and glassfiber filter (such as Whatman GF/F) so the sample is filtered before entering the sample bottle, followed by freezing until analysis. Note that glass bottles may break upon freezing, even if adequate space is left in the bottle for expansion. If freshwater samples are filtered, be sure to first flush filters with sufficient sample water, since glassfiber filters initially adsorb ammonium from freshwater samples (Eaton and Grant 1979). If samples cannot be filtered in the field, it may be better to omit filtration and freeze as soon as possible instead of exposing the sample to contamination in the laboratory during filtration prior to freezing.

Background fluorescence and matrix effects

Two additional issues that we will address are background fluorescence (BF) and matrix effects (ME). BF results from substances in the sample that autofluoresce; this fluorescence must be subtracted from observed fluorescence in order to determine fluorescence resulting from ammonium in the sample. ME are caused by substances in the sample that alter the intensity of the fluorescence caused by orthophthaldialdehyde (OPA) reacting with ammonium in the sample. Although ME are often small (particularly when using Protocol B), they can be substantial in some samples and must be considered. We present methods for dealing with BF and ME that allow accurate correction of results.

Reagents

The method uses a stable working reagent (WR) that is common to Protocols A and B. The active ingredient in the WR is OPA. Although OPA is also used to measure amino acids, when combined

© 1999 NRC Canada

with sodium sulfite (instead of mercaptoethanol), it loses sensitivity to amino acids and thus is essentially specific to ammonium (Kérouel and Aminot 1997). The other two reagents in the WR are a borate buffer and sodium sulfite. The borate buffer, without OPA or sodium sulfite, is used to evaluate sample BF (see Background fluorescence and matrix effects section). Only freshly drawn ultrapure deionized (DI) water should be used to prepare standards and solutions. Since relatively large quantities of WR are used (typically 10–20 mL·sample^{-1}), we make it in large batches; the recipe below makes ~2 L.

Sodium sulfite solution

Add 1 g of sodium sulfite (we use Sigma S-4672) to 125 mL of DI water. The resulting solution is stable for ~1 month when stored at room temperature in a glass bottle.

Borate buffer solution

Add 80 g of sodium tetraborate (we use Sigma S-9640) to 2 L of DI water. Stir or shake thoroughly to dissolve.

OPA solution

Add 4 g of OPA (we use Sigma P-1378) to 100 mL of ethanol (use a high-grade ethanol because impurities in ethanol can autofluoresce; we have used both Carlo Erba and Fisher brands with good results). OPA is light sensitive, so it should be protected from light while dissolving in ethanol and stored in the dark.

WR

In a large (>2 L) brown polyethylene bottle, mix 2 L of borate buffer solution, 10 mL of sodium sulfite solution, and 100 mL of OPA solution. Ideally, allow the WR to "age" for 1 day or more prior to use because its blank will decrease over time. The resulting WR is stable for at least 3 months when stored in the dark at room temperature. To summarize, the final WR should contain the chemicals at the following concentrations: borate buffer (40 g·L^{-1}, 21 mM in the WR), sodium sulfite (40 mg·L^{-1}, 0.063 mM in the WR), and OPA in ethanol (50 mL·L^{-1} in the WR).

Analytical equipment

We use a Turner Designs 10-AU field fluorometer equiped with optical kit No. 10-303. The optical kit consists of a near ultraviolet mercury vapor lamp, a 350-nm interference excitation filter with a 25-nm bandpass, a 410- to 600-nm combination emission filter, and a 1:75 attenuator plate to reduce signal strength. Samples are manually introduced into the fluorometer in borosilicate test tubes, which are used once only. There is no need to preclean test tubes. The sample is read immediately after entering the test tube; thus, any ammonium contamination that might be in the test tube does not have time to react before being read. Alternatively, a single test tube can be reused by rinsing thoroughly with DI water between samples.

Standard preparation

Preparation of standards is nontrivial. Our recommended method for making standards is out of the ordinary and therefore requires explanation. Since we favor adding the WR immediately after the sample is collected in the field, standard reactions should also be started in the field unless the period between sample collection and returning to the laboratory is very short.

Protocol A standards

Take a set of sample bottles to the field filled with precisely 80 mL of DI water. Also take some ammonium stock solution (we typically use 50 μmol·L^{-1}, but more or less concentrated solutions may be preferable in some cases) and an adjustable pipette (we use a 100- to 1000-μL Eppendorf). During the period when samples are collected, standards are made by pipetting stock solution into the sample bottles filled with DI water, and then the WR is added. When calculating standard concentration, one must account for the volume of the stock solution added to the DI water.

We obtain better results using the above protocol than when we prepare standards in volumetric flasks and then transfer to sample bottles, perhaps for the following reasons. First, every time the sample is transferred between glassware, contamination can occur. Second, in freshwater standards and samples, ammonium may bind to the surface of glass; thus, the solution coming out of the volumetric flask may be less than expected (Kérouel and Aminot 1997). By preparing the standard in the bottle when the reagents are added, any ammonium from the water sample that has bound to the bottle will participate in the reaction.

Protocol B standards

Standards are prepared similarly to Protocol A, except that 2.5 mL of DI water and a quantity of ammonium stock solution are added to the sample containers that are already filled with 10 mL of WR. For example, addition of 2.5 mL of DI water and 100 μL of 50 μmol ammonium·L^{-1} stock solution to the WR-filled sample bottles results in a 1.923 μmol ammonium·L^{-1} standard.

Basic procedure — protocol A

Supplies and preparation

1. Acid-washed sample bottles, ~125 mL volume. Extra bottles are needed for determination of the BF and ME of selected samples.
2. WR, enough for 20 mL for each sample and standard.
3. Small graduated cylinder to measure 20 mL of WR.
4. Several sample bottles filled with 80 mL of DI water. These bottles will be used to make standards.
5. Ammonium stock solution.
6. Adjustable pipette and pipette tips to add ammonium stock solution to standards and ME samples (see Background fluorescence and matrix effects section).

Field procedures

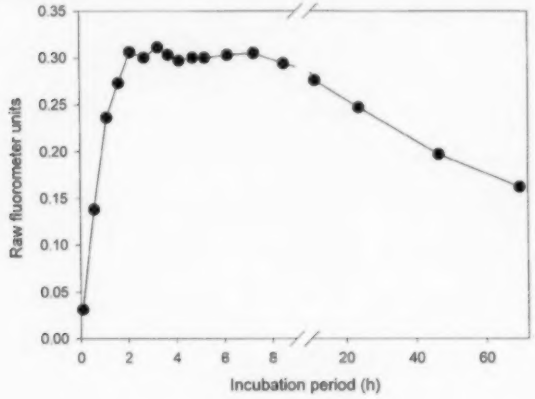
1. Rinse bottle once with sample water and then fill to the 80-mL mark.
2. Add 20 mL of WR and shake and then store in the dark at ambient temperature.
3. Prepare standards (see Protocol A standards section) by adding ammonium stock solution and WR. Standards should be made during the period when samples are collected. If sample collection takes >4 h, more than one set of standards may be needed in order that samples and standards will be at a similar stage of reaction.
4. If the ME is unknown, collect additional samples from selected locations (try to span the range of sample types to be collected; for example, encompass the full salinity range). Amend these samples with the same amount of ammonium stock solution as was used for an intermediate concentration standard. The response of the amended samples will be compared with the standards in order to determine the ME (see Background fluorescence and matrix effects section).
5. If background fluorescence is unknown or variable, collect samples from selected locations for BF determination. The borate buffer solution can be added upon return to the laboratory, since no incubation period is necessary.

Laboratory procedures

1. After incubating for at least 2–3 h, pour samples and standards into test tubes and immediately read on the fluorometer. More than one sample per minute can typically be read.
2. Calculate sample ammonium concentration. The procedure for calculating a sample's concentration involves four steps:

© 1999 NRC Canada

Fig. 2. Time course of the reaction at 22°C. Sample ammonium concentrations were 0.2 μmol·L^{-1}.



(*i*) measuring the raw fluorescence of the sample ($F_{sample_{obs}}$), (*ii*) subtracting BF ($F_{sample_{BF}}$), (*iii*) correcting for any ME, and (*iv*) calculating sample concentration using the standard regression (see Background fluorescence and matrix effects section for details of calculations).

Basic procedure — protocol B

Supplies and preparation

1. Sample containers filled with 10 mL of WR. We generally use Wheaton 20-mL HDPE scintillation vials as sample "bottles." Instead of washing the vials, we find it easier to incubate them with WR for at least 1 day and then discard the WR and refill with precisely 10 mL of WR. The incubation process removes any ammonium that may be present in the sample container. Take additional sample containers filled with WR for standards and ME samples.
2. Pipette to add 2.5 mL of sample or DI water into sample bottles.
3. Ammonium stock solution and an adjustable pipette for adding stock to standards.
4. Empty bottles for collecting samples for BF analysis.

Field procedures

1. Using a pipette or other accurate measuring device, add 2.5 mL of sample to a sample container that has been filled with 10 mL of WR. Shake and store in the dark.
2. Prepare standards (see Protocol B standards section) during the period when samples are being collected. As noted previously, if sample collection takes several hours, more than one set of standards may be needed in order that samples and standards will be at a similar stage of reaction.

Laboratory procedures

Same as for Protocol A.

Results and discussion

Characterization of the reaction

The kinetics of the reaction were investigated to determine the required incubation period, and spectral characteristics of the fluorescent compound were evaluated to assess optimal excitation and emission wavelengths. The tests were made using Protocol A but are equally applicable to Protocol B.

Time course of reaction

To investigate the time course of the reaction, 18 standards (0.2 μmol ammonium·L^{-1}) were prepared simultaneously and analyzed at intervals over a 3-day period until all standards had been analyzed (Fig. 2). The reaction reached maximum fluorescence after ~2 h, remained relatively stable for several more hours, and then gradually declined. Ideally samples should be read during the period of plateau, ~2–8 h after mixing sample and WR. If for logistical reasons this is not feasible, it is still possible to get good results even after fluorescence has begun to decline, since samples and standards degrade at the same rate.

We also tested the effect of reducing or elevating incubation temperature on the time course of the reaction. It was possible to substantially speed the reaction by incubating in a 40°C water bath or slow it by placing the reacting samples on ice. In general, however, we favor reaction at ambient temperature (generally 18–25°C in our case) unless it is not feasible to wait 2–3 h for the fluorescence to plateau. Note that since the speed of the reaction is temperature dependent, samples and standards should begin at approximately the same temperature. This is particularly true for Protocol A, since sample volumes are large and there is little dilution with WR. In the case where samples and standards begin at very different temperatures, it is best to allow additional incubation time (>4 h) so that both are fully reacted before reading their fluorescence.

Excitation and emission spectra

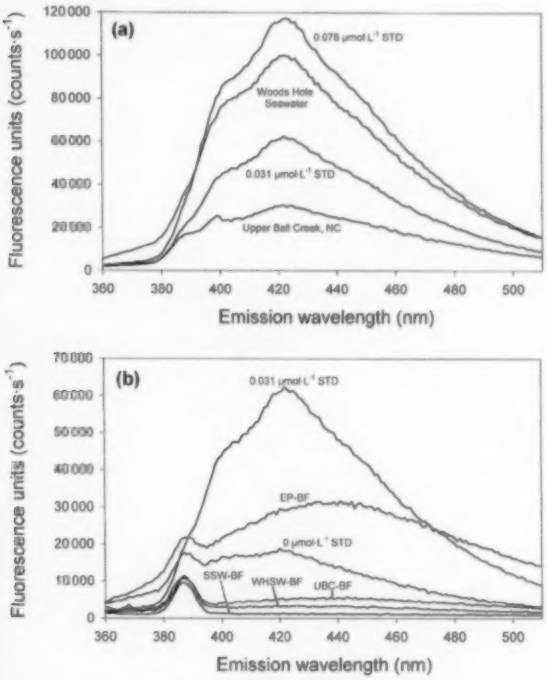
In order to choose optimal excitation and emission wavelengths for the method, we measured spectral characteristics of samples and standards using a Photon Technology International scanning fluorometer. We measured the excitation spectra for a 12.5 μmol ammonium·L^{-1} standard and found that maximum emission occurred when the excitation wavelength was ~360 nm. For subsequent scans, we used an excitation bandwidth of 340–360 nm.

For both samples and standards, maximum emission was at ~420 nm, but the peaks were rather broad, ranging from ~380 to >500 nm (Fig. 3*a*). We concluded that excitation below 380 nm and quantifying emissions above 400 nm was appropriate. We find that our filter set (350-nm peak, 25-nm bandpass excitation, 410–600 nm emission) works well, but other combinations within the prescribed range should also be adequate.

We had hoped to be able to minimize interference from BF (sample autofluorescence) by selecting a narrow-bandwidth emission filter that separated OPA-ammonium fluorescence from BF. To quantify BF, we add borate buffer and measure fluorescence. This is done to account for dilution by the WR and because fluorescence is pH dependent. Unfortunately, overlap between OPA-ammonium fluorescence and BF is relatively large (Fig. 3*b*), so complete separation with a narrow-bandwidth emission filter is not possible. However, by focusing around the peak OPA-ammonium fluorescence of ~420 nm, the signal to noise ratio could be increased. Note that in most cases tested, BF is rather small, generally less than the signal of the 0 μmol·L^{-1} standard, which itself fluoresces because the OPA in the WR has a small amount of fluorescence. The peak observed at

© 1999 NRC Canada

Fig. 3. (*a*) Emission spectra for standards and samples after incubation with WR for 4 h. Peak fluorescence for both samples and standards was 421–423 nm. (*b*) Emission spectra for standards (with WR) and samples (without WR) to determine if appropriate filter selection might allow OPA-ammonium fluorescence to be separated from sample BF. EP, Eel Pond in Woods Hole, which was brown in color when these samples were taken due to storm runoff; SSW, Sargasso seawater; WHSW, Woods Hole seawater; UBC, Upper Ball Creek, a small stream in North Carolina.



~385 nm (Fig. 3*b*) is due to the Raman signal of water and is avoided by selecting an emission filter with a wavelength >400 nm.

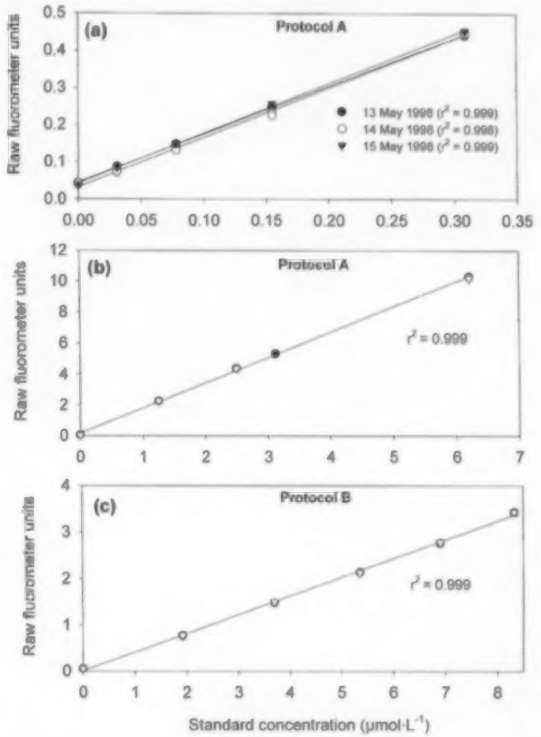
Standards

Using Protocol A, we made 0, 0.031, 0.078, 0.155, and 0.309 μmol ammonium·L^{-1} standards on 3 consecutive days. Duplicate standards of each concentration were run on the first 2 days, and a single replicate of each concentration was done on the third day. In all cases, the resulting linear regressions were good, with little variability among days (Fig. 4*a*). There were no outliers. The signal strength of the lowest standard (0.031 μmol·L^{-1}) was unambiguous, approximately double the blank fluorescence. The reagent blank (or possibly ammonium contamination in our DI water) was equivalent to ~0.03 μmol·L^{-1}.

We also ran standards up to 6.2 μmol·L^{-1} using Protocol A and fluorescence remained linear (Fig. 4*b*). In other experiments, we ran samples up to 25 μmol·L^{-1} and could have adjusted the instrument to read even higher concentrations.

Protocol B also yielded good results (Fig. 4*c*). We are able to analyze ammonium in samples up to ~50 μmol·L^{-1} without dilution or adjusting the sensitivity of the fluorometer

Fig. 4. Standard curves. (*a*) Low-concentration ammonium standards run on 3 consecutive days using Protocol A. On the first two dates, duplicates were done for each concentration standard, whereas on the third date, there was only one standard per concentration. (*b*) Higher concentration ammonium standards analyzed using Protocol A. Duplicates of each concentration were analyzed. (*c*) Standards analyzed using Protocol B. Duplicates of each concentration were analyzed.



using Protocol B. Below ~0.5 μmol·L^{-1}, however, variability increased, so we recommend Protocol A for samples with less than ~0.5 μmol·L^{-1}.

Background fluorescence and matrix effects

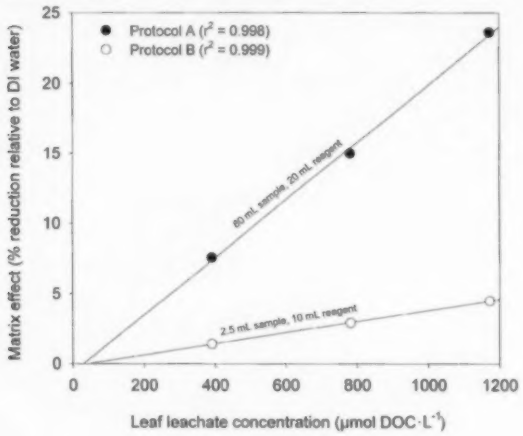
A sample's BF is determined by mixing the sample with borate buffer instead of WR. No incubation period is necessary. The fluorescence of the sample when combined with borate buffer ($F_{\text{sample}_{\text{BF}}}$) is subtracted from its fluorescence when incubated with WR ($F_{\text{sample}_{\text{obs}}}$) to determine the sample's ammonium fluorescence ($F_{\text{sample}_{\text{NH}_4}}$):

$$(1) \qquad F_{\text{sample}_{\text{NH}_4}} = F_{\text{sample}_{\text{obs}}} - F_{\text{sample}_{\text{BF}}}.$$

ME are caused by substances in the sample such as sea salt or dissolved organic matter that make the sample behave differently than standards. ME are an issue for most methods, including the common indophenol blue technique for measuring ammonium (Loder and Glibert 1977; Stewart and Elliott 1996). To determine the magnitude of matrix effects, we amended samples and standards with the same amount of ammonium and compared the response:

© 1999 NRC Canada

Fig. 5. Quantification of ME with different amendments of leaf litter leachate. ME is plotted as percent reduction of signal in amended samples relative to DI water standards.



$$(2)\qquad \mathrm{ME} = \left\{ \frac{[(F_{\mathrm{std}_{\mathrm{spike}}} - F_{\mathrm{std}_{\mathrm{zero}}}) - (F_{\mathrm{sample}_{\mathrm{spike}}} - F_{\mathrm{sample}_{\mathrm{obs}}})]}{(F_{\mathrm{std}_{\mathrm{spike}}} - F_{\mathrm{std}_{\mathrm{zero}}})} \right\} \times 100\%.$$

If there were no ME, samples and standards would respond identically. More typically, the response is somewhat attenuated in samples relative to DI water standards, which must be considered when calculating sample concentration.

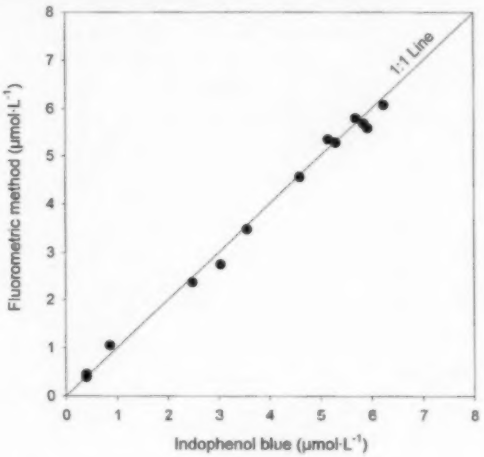
When analyzing seawater samples using standards made in DI water, the ME is ~14–17% when using Protocol A but is reduced to ~3–5% for Protocol B. That is, a 1 μmol ammonium·L^{-1} amendment added to a seawater sample using Protocol A fluoresces only ~85% as much as a 1 μmol·L^{-1} amendment of DI water. To investigate the ME associated with dissolved organic matter, we amended DI water with leaf litter leachate (to dissolved organic carbon concentrations of 391, 782, and 1172 μmol·L^{-1}) and quantified the ME (Fig. 5). At the highest dissolved organic carbon concentration (1172 μmol·L^{-1}), the ME was ~24% using Protocol A but only 4.5% for Protocol B. Thus, Protocol B has the advantage of requiring a smaller ME correction but is less well suited for samples with very low ammonium concentrations because of reduced sensitivity. BF and ME estimates are combined to determine corrected sample fluorescence ($F_{\mathrm{sample}_{\mathrm{corr}}}$) (eq. 3). The value of $F_{\mathrm{sample}_{\mathrm{corr}}}$ is entered into the standard regression to compute the sample's ammonium concentration:

$$(3)\qquad F_{\mathrm{sample}_{\mathrm{corr}}} = F_{\mathrm{sample}_{\mathrm{NH_4}}} + \left[F_{\mathrm{sample}_{\mathrm{NH_4}}} \left(\frac{\mathrm{ME}}{100} \right) \right].$$

Comparison with indophenol blue method

We compared the fluorometric method with the indophenol blue method using marine and freshwater samples, some amended with ammonium. Results obtained from the two methods were comparable (Fig. 6). Protocol B was used for the fluorometric method, and the indophenol method followed Solorzano (1969) using 3-mL samples.

Fig. 6. Comparison of fluorometric and indophenol blue methods. Marine and freshwater samples were measured simultaneously with both methods. The similarity of results demonstrates the comparability of the two methods.



Field samples

The fluorometric method (Protocols A and B) was used on a variety of samples, both marine and freshwater (Table 1). BF ($F_{\mathrm{sample}_{\mathrm{BF}}}$) ranged from 1.6 to ~30% of observed sample fluorescence ($F_{\mathrm{sample}_{\mathrm{obs}}}$), and ME ranged from 4 to 18%. ME for Protocol B did not exceed 6%. The relatively high BF of the Parker River sample is not surprising, since the sample was highly colored with dissolved organic matter. Moreover, while the BF for some samples seems rather high, this is largely an artifact of the low ammonium concentrations of the samples. When converted to ammonium concentration equivalents, the observed BF ranged from only ~0.007 to 0.13 μmol·L^{-1}. Ammonium concentrations for all samples were <0.5 μmol·L^{-1}, and replication was good in all cases. Samples of Woods Hole seawater and Parker River water were analyzed using both protocols, and results differed by 0.03–0.05 μmol·L^{-1}. Although replication was good using both protocols, in general, we are more confident in analyzing samples with less than ~0.5 μmol·L^{-1} using Protocol A. Since the Sargasso seawater sample contained no measurable ammonium, standards could be made in the Sargasso seawater, which would greatly reduce the ME when analyzing seawater samples.

We also tested the method on a series of samples from the Kuparuk River and Toolik Lake, at the site of the Arctic LTER project in northern Alaska. The stream samples were taken along a 4.3-km reach of the river that has been subject to a long-term phosphorus fertilization experiment (Peterson et al. 1993, 1997). Primary production is stimulated in the phosphorus-fertilized reach, and consequently, we might expect a downstream decline in ammonium concentration. Ammonium concentrations are always low, and prior to summer 1998 when we began using the fluorometric ammonium method, any such pattern that might have been present was obscured by analytical noise. However, ammonium analy-

© 1999 NRC Canada

Table 1. Analysis of ammonium concentrations in a variety of marine and freshwater samples using both variations of the method (Protocols A and B).

Sample	Protocol	Observed fluorescence ($F_{sample_{obs}}$) (raw units ± SE)	BF ($F_{sample_{BF}}$) (raw units ± SE)	Sample NH_4 fluorescence ($F_{sample_{NH_4}}$) (raw units ± SE)	ME (relative to DI water) (%)	Corrected sample fluorescence ($F_{sample_{cor}}$) (raw units ± SE)	Sample concentration (µmol·L^{-1} ± SE)[a]
Woods Hole seawater	A	0.611±0.0013	0.010±0.0000	0.601±0.0013	15	0.692±0.0015	0.43±0.001
Woods Hole seawater	B	0.179±0.0007	0.005±0.0003	0.174±0.0007	4	0.182±0.0003	0.40±0.001
Parker River, Mass.	A	0.679±0.0038	0.178±0.0003	0.501±0.0038	18	0.591±0.0045	0.36±0.003
Parker River, Mass.	B	0.202±0.0012	0.058±0.0009	0.144±0.0012	6	0.153±0.0011	0.31±0.003
Sargasso seawater	A	0.027±0.0022	0.004±0.0000	0.023±0.0022	17	0.027±0.00260	0.00±0.002
Upper Ball Creek, N.C.	A	0.066±0.0010	0.020±0.0005	0.046±0.0010	5	0.049±0.0013	0.01±0.001

Note: Error terms represent SE of three replicates.

[a]For Protocol A standards, the slope of the regression equation was 1.556, the y-intercept (blank) was 0.029, and the r^2 was 0.9998; for Protocol B standards, the slope of the regression equation was 0.3489, the y-intercept (blank) was 0.043, and the r^2 was 0.9995.

Fig. 7. Ammonium concentrations measured in samples collected along the Kuparuk River, Alaska, the site of a long-term phosphorus addition experiment (phosphate added at 0.3 km). Samples were collected 20 July 1998. As expected, ammonium concentrations declined downstream of the phosphorus addition site because primary production (and nutrient uptake) was stimulated by the phosphorus addition.

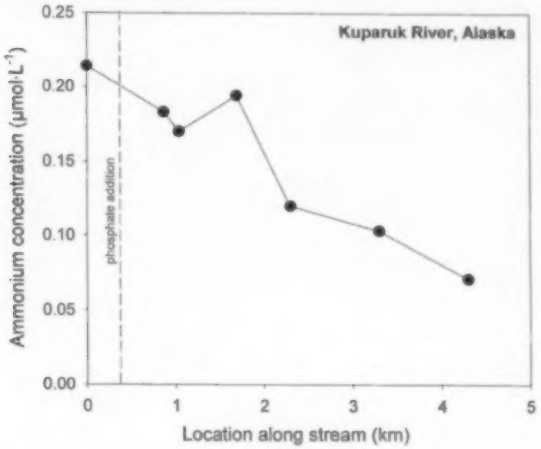
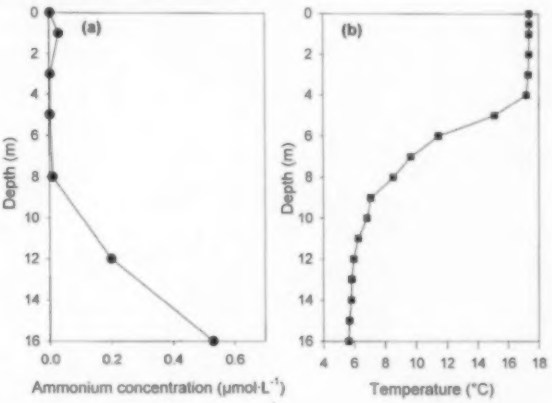


Fig. 8. Toolik Lake, Alaska, (*a*) ammonium and (*b*) temperature profiles from 22 July 1998. Ammonium concentrations were extremely low in the epilimnion and metalimnion but rapidly increased in the hypolimnion.



sis using the fluorometric method demonstrated the expected pattern; ammonium concentration declined from ~0.2 µmol·L^{-1} at the upstream end of the reach to 0.07 µmol·L^{-1} ~4 km downstream (Fig. 7).

The ability to measure extremely low ammonium concentrations using the fluorometric method also increased our understanding of nitrogen cycling in the oligotrophic Toolik Lake (Fig. 8). In the epilimnion, ammonium concentrations were generally <0.02 µmol·L^{-1} but increased to almost 0.6 µmol·L^{-1} in the hypolimnion. As was the case in the Kuparuk River, this pattern was previously much less apparent because concentrations were typically too low for us to measure using the indophenol method.

© 1999 NRC Canada

Conclusions

We have used the method on a variety of samples from diverse sites and in all cases have been pleased with its performance. We presented two variations of the method (Protocols A and B) and should note that other sample volumes and sample to reagent ratios can be used; for example, 10 mL sample and 10 mL reagent may be optimal for some applications. In our first field season using the fluorometric method at the Arctic LTER site (summer 1998), the ability to measure ammonium at low concentrations with good accuracy provided insight into the functioning of the Kuparuk River and Toolik Lake ecosystems that previously was obscured by analytical noise. The fluorometric ammonium method has also allowed us to improve our measurements of the isotopic composition of ammonium that we conduct at the Arctic LTER site and other sites (Holmes et al. 1998, 1999; Mulholland et al. 2000), as accurate ammonium concentration measurements are critical for these analyses. We conclude that the method is broadly applicable, relatively simple, and will advance our understanding of ecosystem processes by providing more reliable ammonium concentration measurements.

Acknowledgements

This research was supported by grants NSF-DEB-9407829, NSF-OPP-9818199, NSF-DEB-9810222, NSF-DEB-9211775, NSF-OCE-9726921, and NSF-OPP-9524740. We thank Jim McClelland (University of East Anglia, England), Deana Erdner (University of Norwich, England), Gabrielle Tomasky and Ivan Valiella (Boston University Marine Program), and Kama Thieler, Nat Weston, Inger Kappel-Schmidt, Annika Nordin, Jane Tucker, and Dave Vasilou for testing the method during development. John Hobbie, Neil Bettez, Hap Garritt, Anne Giblin, Gabrielle Tomasky, Wil Wollheim, and two anonymous reviewers provided constructive critiques of the manuscript. We also thank Heather Bush and Rob Ellison of Turner Designs for assistance and providing various trial filters and Steven Sczekan of Photon Technology International for access to and assistance with their scanning spectrofluorometer.

References

Aminot, A., Kirkwood, D.S., and Kérouel, R. 1997. Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C5 questionaire. Mar. Chem. **56**: 59–75.

Brzezinski, M.A. 1987. Colorimetric determination of nanomolar concentrations of ammonium in seawater using solvent extraction. Mar. Chem. **20**: 277–288.

Catalano, G. 1987. An improved method for the determination of ammonia in seawater. Mar. Chem. **20**: 289–295.

Eaton, A.D., and Grant, V. 1979. Sorption of ammonium by glass frits and filters: implications for analyses of brackish and freshwater. Limnol. Oceanogr. **24**: 397–399.

Gardner, W.S., and St. John, P.A. 1991. High-performance liquid chromatographic method to determine ammonium ion and primary amines in seawater. Anal. Chem. **63**: 537–540.

Garside, C., Hull, G., and Murray, S. 1978. Determination of submicromolar concentrations of ammonia in natural waters by a standard addition method using a gas-sensing electrode. Limnol. Oceanogr. **23**: 1073–1076.

Genfa, Z., and Dasgupta, P.K. 1989. Fluorometric measurement of aqueous ammonium ion in a flow injection system. Anal. Chem. **61**: 408–412.

Gibb, S.W., Mantoura, R.F.C., and Liss, P.S. 1995. Analysis of ammonia and methylamines in natural waters by flow injection gas diffusion coupled to ion chromatography. Anal. Chim. Acta, **316**: 291–304.

Goyal, S.S., Rains, D.W., and Huffaker, R.C. 1988. Determination of ammonium ion by fluorometry or spectrophotometry after online derivation with *o*-phthaldehyde. Anal. Chem. **60**: 179–179.

Holmes, R.M., McClelland, J.W., Sigman, D.M., Fry, B., and Peterson, B.J. 1998. Measuring $^{15}N\text{-}NH_4^+$ in marine, estuarine, and fresh waters: an adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Mar. Chem. **60**: 235–243.

Holmes, R.M., Peterson, B.J., Deegan, L., Hughes, J., and Fry, B. 1999. Nitrogen biogeochemistry in the oligohaline zone of a New England estuary. Ecology. In press.

Kérouel, R., and Aminot, A. 1997. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. Mar. Chem. **57**: 265–275.

Loder, T.C., and Glibert, P.M. 1977. Blank and salinity corrections for automated nutrient analysis of estuarine and sea waters. Advances in Automated Analysis: Technicon International Congress 1976, New York. Mediad Inc., Tarrytown, N.Y.

Mulholland, P.J., Tank, J.L., Sanzone, D.M., Wollheim, W.M., Peterson, B.J., Webster, J.R., and Meyer, J.L. 2000. Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. Ecol. Monogr. **70**(3). In press.

Patton, C.J., and Crouch, S.R. 1977. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem. **49**: 464–469.

Peterson, B.J., Deegan, L., Helfrich, J., Hobbie, J.E., Hullar, M., Moller, B., Ford, T.E., Hershey, A., Hiltner, A., Kipphut, G., Lock, M.A., Fiebig, D.M., McKinley, V., Miller, M.C., Vestal, J.R., Ventullo, R., and Volk, G. 1993. Biological responses of a tundra river to fertilization. Ecology, **74**: 653–672.

Peterson, B.J., Bahr, M., and Kling, G.W. 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. Can. J. Fish. Aquat. Sci. **54**: 2361–2367.

Schierup, H.-H., and Riemann, B. 1979. Effects of filtration on concentrations of ammonia and orthophosphate from lake water samples. Arch. Hydrobiol. **86**: 204–216.

Solorzano, L. 1969. Determination of ammonium in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. **14**: 799–801.

Stewart, B.M., and Elliott, P.A. W. 1996. Systematic salt effects in the automated determination of nutrients in seawater. Water Res. **30**: 869–874.

© 1999 NRC Canada

Nitrogen–phosphorus relationship in high mountain lakes: effects of the size of catchment basins

Rafael Morales-Baquero, Presentación Carrillo, Isabel Reche, and Pedro Sánchez-Castillo

Abstract: We analyzed the changes in epilimnetic total nitrogen (TN), total phosphorus (TP), dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP) in 31 small high-mountain lakes in the Sierra Nevada (Spain) during an annual cycle, just after the spring thaw, and in the middle of the growing season. Chlorophyll *a*, TN, and TP increased, whereas the TN:TP ratio fell substantially between the two periods, reaching values generally between 25 and 10 (by weight). On the contrary, DIN, SRP, and DIN:SRP ratios were similar for both periods in each lake. DIN:SRP ratios generally ranged from 5 to 20 (by weight). This ratio was low in the lakes with small catchment areas and increased progressively with catchment basin size. A regression analysis for the smallest catchments showed that chlorophyll *a* concentrations were not accounted for by variability in TP concentration. The results obtained are discussed in relation to the influence of episodes of Saharan dust, rich in P, reaching the Southern Mediterranean area.

Résumé : Nous avons analysé les variations de l'azote total (NT), du phosphore total (PT), de l'azote inorganique dissous (NID) et du phosphore soluble réactif (PSR) de l'épilimnion de 31 petits lacs de haute montagne de la Sierra Nevada (Espagne) pendant un cycle annuel, tout juste après le dégel du printemps et au milieu de la saison de croissance. Les concentrations de chlorophylle-*a*, de NT et de PT ont augmenté tandis que le rapport NT:PT a diminué de façon appréciable entre les deux périodes pour atteindre des valeurs se situant généralement entre 25 et 10 (en poids). Au contraire, les concentrations de NID et de PSR de même que le rapport NID:PSR étaient semblables au cours des deux périodes et dans chaque lac. Le rapport NID:PSR se situait généralement entre 5 et 20 (en poids). Le rapport était faible dans les lacs à bassin versant peu étendu et augmentait progressivement avec la superficie du bassin. Une analyse de régression portant sur les bassins les plus petits a montré que les concentrations de chlorophylle-*a* ne se reflétaient pas dans la variabilité de la concentration de PT. Les résultats obtenus sont traités dans le contexte de l'influence de vents en provenance du Sahara qui transportaient de la poussière, riche en P, et atteignait le sud de la Méditerranée.

[Traduit par la Rédaction]

Introduction

The nitogen to phosphorus (N:P) ratio in lake water can influence phytoplankton structure and biomass (McCauley et al. 1989; Molot and Dillon 1991). This ratio is dependent on external sources of N and P (runoff, atmospheric precipitation, groundwater, etc.) and on internal processes (phytoplankton uptake, sediment release, recycling by heterotrophs, etc.). Among external sources, atmospheric inputs have been recognized as a major supply of nutrients, mainly P, for many lakes located in undeveloped lands with small catchments (Peters 1977; Rigler and Peters 1995). The importance of N inputs from the atmosphere, however, has progressively increased as a result of industrial development (Henriksen and Brakke 1988).

Downing and McCauley (1992) established a close correlation between total N to total P (TN:TP ratios) in lake water and ratios in inputs for a wide gradient of trophic conditions throughout the world and hypothesized that "...oligotrophic lakes in undeveloped areas that have small catchment basins relative to their area should have lower TN:TP ratios because precipitation has lower N:P than export from undeveloped lands." The N:P ratios of atmospheric precipitation are not always lower than those of export from undeveloped areas (Dillon et al. 1991); the hypothesis was meant to refer to average conditions globally. In general, the N:P ratios of runoff from unfertilized land are higher than those in precipitation for a homogeneous geographical area. Consequently, as lake catchment area increases, a progressive increase in the N:P ratios of external inputs is expected. Moreover, if the N:P ratio of the precipitation is low enough, P limitation of phytoplankton should become less likely as lake catchment area decreases. Since the dissolved inorganic N to soluble reactive P (DIN:SRP) ratio has proved to be a sensitive index for phytoplankton nutrient supply in lakes (Morris and Lewis 1988), a negative relationship between this ratio and catchment area could be expected.

Received November 2, 1998. Accepted July 6, 1999.
J14872

R. Morales-Baquero[1] and P. Carrillo. Instituto del Agua, Universidad de Granada, 18071 Granada, Spain.
I. Reche. Departamento de Biología Animal y Ecología, Universidad de Granada, 18071 Granada, Spain.
P. Sánchez-Castillo. Departamento de Biología Vegetal, Universidad de Granada, 18071 Granada, Spain.

[1]Author to whom all correspondence should be addressed.
e-mail: rmorales@goliat.ugr.es

© 1999 NRC Canada

High mountain lakes seem to be ideal places to test and contrast the above-mentioned hypothesis and related arguments. They are normally located in remote areas, hardly affected by human activity, and have small catchment basins and severe nutrient limitation. However, some authors (Kopácek et al. 1995) found much higher TN:TP ratios than the world average calculated by Downing and McCauley (1992) in lakes of central European mountains subject to strong and regular atmospheric N deposition linked to industrial activity. They concluded that an increase in the TN:TP ratio in geographically diverse high mountain lakes of the Northern Hemisphere is expected due to the increasing N inputs of industrial activity (i.e., increased nitric acid in rainfall).

The aim of this study was to examine lake TN:TP and DIN:SRP ratios in relation to catchment size in a group of small high mountain lakes in the Sierra Nevada (Spain). Low atmospheric N:P ratios are expected in this geographical region because it is far from areas of heavy industry. Moreover, every year, the region is exposed to long massive airborne plumes of desert dust from the Sahara Desert (Moulin et al. 1997). The dust has a substantial P content in comparison with N (Talbot et al. 1986). Sampling took place just after ice thaw and later, in the middle of the growing season, when plankton communities were well developed.

Materials and methods

Site description

Around 50 small glacially formed lakes are present in the Sierra Nevada (Spain) (36°55′–37°15′N, 2°31′–3°40′W; maximum altitude 3482 m above sea level) at elevations of roughly 2800–3100 m above sea level. The predominant substrate in the catchment basins is siliceous rocks, mainly mica–schist with graphite and mica–schist with feldspar. The soils are very poorly developed and do not support agriculture or forestry use. The vegetation is restricted to some sparse meadows. Lake size and depth vary with annual rainfall. In this study, surface areas ranged from 0.01 to 2.1 ha and maximum depths from 0.3 to 8 m (Table 1). Lakes normally remain ice covered from October to June. Some of them have outlets that may disappear as the ice-free period advances. Lake waters are relatively soft: conductivity ranged from 5 to 77 $\mu S \cdot cm^{-1}$, total alkalinity from 50 to 400 $\mu equiv. \cdot L^{-1}$, and pH from 6.5 to 9.5 (mean values at the time of this study).

The assumption of low N:P ratios in atmospheric inputs in this area is consistent with the preliminary information on the TN:TP ratios of snow samples, which varied from 4.3 to 15.9 (average 8.8) during the winter of 1996 (M. Villar-Argaiz, Depatamento de Biología Animal y Ecología, Universidad de Granada, 18071 Granada, Spain, personal communication).

Phytoplankton is dominated by nanoplanktonic species (Sánchez-Castillo et al. 1989), and the dominant zooplankton species are *Mixodiaptomus laciniatus* and *Diaptomus cyaneus* among copepods, *Daphnia pulicaria* among cladocerans, and *Hexarthra bulgarica* among rotifers (Cruz-Pizarro 1983; Morales-Baquero et al. 1989). The lakes are fishless.

Sampling and analysis

A total of 31 lakes were studied; samples were taken at the beginning and in the middle of the ice-free period. We tried to collect the samples from all the lakes in the shortest time period possible. The first sampling took place over a 12-day period between 15 and 27 July 1991, and the second was realized over a 13-day period between 21 August and 3 September in the same year. Hereafter, these periods will be referred to as "July" and "August," respectively. Because some of the lakes dried up during the summer, and some samples were lost, the number of lakes that we could compare between the two periods varied between 20 and 24. Watershed and lake surface sizes were calculated by image analysis from maps (1 : 25 000 scale) and aerial photographs (1:2500 scale), respectively.

Samples were taken at the deepest point of each lake. An equal volume of water was extracted (using a centrifugal electric pump) from four evenly spaced levels of the vertical profile, prefiltered through a 40-μm nytal net to remove zooplankton, and then mixed together. Aliquots for analysis were taken from this mixed sample. Those for chemical analysis were stored in the dark at 4°C and analyzed within 24 h of sampling. Chlorophyll *a* (Chl *a*) was determined by filtering 1 L of water through a GF/C filter immediately after collection. The filter was placed in a glass vial, 10 mL of 95% methanol was added, and the vial was stored in the dark at 4°C during transport. The vial was then frozen at –10°C for 24 h and the extract was measured and corrected for pheopigments using an Hewlett-Packard scanning spectrophotometer. For the determination of SRP, filtered aliquots of the water samples (0.45-μm pore size Sartorius filter) were analyzed by the ascorbic acid method of Murphy and Riley (1962) using a 10-cm path length cuvette (detection limit 0.03 $\mu mol \cdot L^{-1}$ (~1 $\mu g \cdot L^{-1}$)). TP was determined as SRP after the digestion of unfiltered aliquots using a mixture of potassium persulphate and boric acid at 120°C for 30 min. NH_4^+-N was determined in 0.45-μm filtrate using the blue indophenol method (Rodier 1990). NO_2^--N and NO_3^--N were determined following the method of Strickland and Parsons (1968) and of sodium salicylate (Rodier 1990), respectively. DIN is the sum of the three former N compounds. TN was determined using the ultraviolet method (APHA 1989) after the digestion of unfiltered aliquots using the same procedure described for TP. Concentrations and elemental ratios are reported by weight. The DIN:SRP ratio has been used as a ratio of nutrient supply to indicate N or P limitation (Morris and Lewis 1988). We used the value 10 (by weight) as a reference to indicate a lower limit of P limitation.

Data were $\log_{10}$ transformed prior to statistical analyses in order to achieve normality and homoscedasticity and to linearise their relationships. Statistical analyses were done using STATISTICA v. 4.0 except for the ANCOVA, which was performed using BMDP-PC90. In multiple regression, the ridge method (Hoerl 1962) for estimating regression coefficients was used when multicollinearity among the independent variables was found.

Results

The Chl *a* content in the lakes increased more than twice, from a mean value of 0.8 $\mu g \cdot L^{-1}$ in July to 1.9 $\mu g \cdot L^{-1}$ in August (Fig. 1). Moreover, the average TN for the lakes increased from 234.4 to 465.5 $\mu g\ N \cdot L^{-1}$, and the average TP increased more than fivefold, from 5.8 $\mu g\ P \cdot L^{-1}$ in July to 30.9 $\mu g\ P \cdot L^{-1}$ in August (Fig. 1). TN and TP concentrations in the lakes increased between July and August as described by the linear equation

$$\Delta TN = 4.388 + 9.078 \cdot \Delta TP$$

$$(r^2 = 0.71,\ p < 0.0001,\ n = 20).$$

TN:TP ratios clearly differed between periods (Fig. 2). In July, TN:TP ratios were relatively high (80% of the lakes had ratios higher than 39 by weight), whereas they suffered a notable reduction in August (ratios ranged from 10 to 25) (Fig. 2). During the first period, no significant correlation existed between TN and TP, whereas during the second pe-

© 1999 NRC Canada

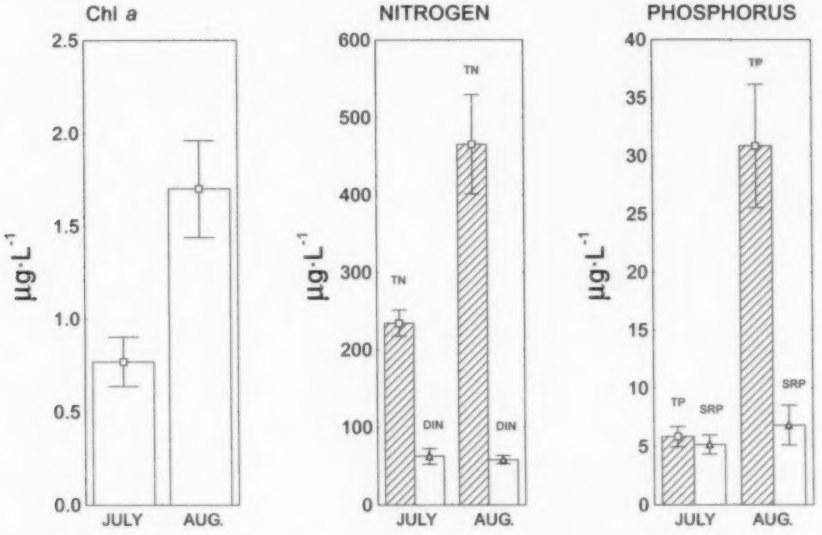
Table 1. Some characteristics of the Sierra Nevada study lakes.

Lake	Location UTM (30S)	Altitude (m above sea level)	Lake surface area (ha)	Catchment area (ha)	Maximum depth (m)	Outflow	DIN ($\mu g\ N \cdot L^{-1}$)	TN ($\mu g\ N \cdot L^{-1}$)	SRP ($\mu g\ P \cdot L^{-1}$)	TP ($\mu g\ P \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)
Virgen 1	VG665008	2950	0.08	25.1	1.3	1	108*	257	5	7	0.4*
Virgen 2	VG664009	2940	0.01	31.2	0.8	1	53	518	4	31	2.2
Dilar 1	VG659007	2870	0.01	71.3	1.0	0	—	117*	1*	2*	0.6*
Dilar 2	VG658006	2860	0.02	71.3	0.8	1	65*	177	16	18	0.3*
Yeguas	VG662013	2880	0.33	50.0	2.5	1	155	594	3	20	2.2
Lanjarón 1	VF646993	2980	0.29	35.8	2.5	1–0	23*	218*	10*	28*	1.1*
Lanjarón 2	VF641991	2940	0.05	7.0	1.0	1	28	276	8	25	1.1
Lanjarón 3	VF636985	2900	0.11	5.8	1.5	0	11	234	9	16	1.9
Mosca	VG723017	2920	0.44	39.7	2.8	1	149	308	4	11	2.1
Majano	VG712003	2900	0.27	72.2	0.8	1–0	62	809	4	33	1.9
Gemela	VG714004	2900	0.07	72.2	0.3	1	39	447	3	21	1.4
Caldera	VG708012	3050	2.10	23.5	7.0	0	101	315*	3	7	0.2
Aguas Verdes	VG674006	3050	0.19	12.8	2.8	1	45	216	8	12	0.6
Larga	VG704017	2790	1.77	29.4	7.0	0	83*	163	2	5	0.3
Rio Seco Superior	VG692008	3040	0.07	4.7	1.5	0	50	435	5	17	0.9
Rio Seco	VG694009	3020	0.42	9.9	2.0	1–0	22	292	3	8	0.5
Rio Seco Inferior	VG697008	3010	0.12	5.8	0.5	1–0	45	930	27	75	2.6
Siete Lagunas 1	VG731016	3060	0.57	10.1	2.5	0	129*	283*	8*	9*	0.9*
Siete Lagunas 2	VG735014	3020	0.34	27.4	3.5	1	68	251	4	13	0.5
Siete Lagunas 4	VG737012	2970	0.19	57.1	0.5	1	93	313	3	10	0.7
Siete Lagunas 5	VG734009	2980	0.18	50.9	2.0	1	65	380	3	16	1.7
Siete Lagunas 7	VG739004	2890	0.53	154.6	0.8	1	106	318	5	15	0.8
Caldereta 1	VG743033	2920	0.23	50.4	3.0	1	99*	255*	7*	7*	0.2*
Caldereta 2	VG745032	2900	0.59	50.4	2.0	0	17*	107*	8*	9*	0.3*
Caldereta 3	VG747033	2890	0.40	50.4	2.5	1	11*	172*	3*	4*	0.8*
Vacares	VG747046	2890	1.10	9.9	8.0	0	61*	112*	2*	3*	0.5*
Juntillas	VG766074	2940	0.11	6.3	1.5	1	42*	200*	2*	3*	0.5*
Mirador	VG691002	2870	0.07	18.2	0.4	1	13*	251*	5*	6*	1.8*
Peñón Negro	VF738983	2820	0.67	28.2	2.0	1	33	479	4	31	4.1
Caballo	VF612968	2840	0.48	10.5	4.0	0	15	161	3	10	0.3
Cuadrada	VF618975	2840	0.24	4.0	5.0	0	21	126*	6	8	0.5

Note: The first three numbers of the UTM coordinates indicate the position to the east and the last three the position to the north. Outflow: 1, lakes with outflow during both sampling periods; 0, lakes without outflow; 1–0, lakes with outflow in the first sampling but not in the second sampling. DIN, TN, SRP, TP, and Chl *a* values are averages for the two sampling periods (those marked with an asterisk are single values).

© 1999 NRC Canada

Fig. 1. Average (±SE) Chl *a*, TN, DIN, TP, and SRP in the Sierra Nevada study lakes during each period studied. Chl *a*, TN, and TP averages are significantly different (respective dependent *t* test: Chl *a* = –3.74 ($p < 0.002$, $n = 20$); TN = –3.23 ($p < 0.005$, $n = 20$); TP = –3.699 ($p < 0.002$, $n = 22$)).



riod, we found a highly significant relationship between the two $\log_{10}$-transformed variables ($r^2 = 0.70$, $p < 0.0001$, $n = 23$).

DIN and SRP concentrations and DIN:SRP ratios did not change between July and August as markedly as TN, TP, and TN:TP ratios (Figs. 1, 2, and 3). Each lake had similar DIN:SRP ratios in July and August. Only two lakes (marked with arrowheads on Fig. 3) had ratios that changed so greatly that they exceeded a value of 10 (our reference threshold for P limitation). Lakes with DIN:SRP ratios lower than 10 showed the highest values of SRP in August, whereas lakes with ratios higher than 10 had SRP values during this period below 3 $\mu g \cdot L^{-1}$ and the highest DIN values.

According to the regression analyses carried out ($\log_{10}$-transformed variables), the TN:TP ratio did not show a significant relationship to catchment area in July ($r^2 = 0.00$, $p = 0.763$, $n = 28$) or August ($r^2 = 0.02$, $p = 0.231$, $n = 23$) or when considering the average of the two periods ($r^2 = 0.00$, $p = 0.309$, $n = 20$). On the contrary, we found significant relationships between catchment basin area and the DIN:SRP ratio for both July ($r^2 = 0.24$, $p = 0.011$, $n = 26$) and August ($r^2 = 0.38$, $p = 0.0015$, $n = 22$), although the explained variance as well as the significance of the analysis increased in August. Since the relationship between catchment basin area and the DIN:SRP ratio may be affected by other factors such as outlets and depth of the lakes, we carried out an ANCOVA on the effect of the presence or absence of outflows on the DIN:SRP ratio, taking catchment basin size and depth as covariables. The result (Table 2) confirmed that catchment size had a significant effect on the DIN:SRP ratio in lakes, with and without outflows and regardless of depth.

In July, only DIN had a significant and positive correlation with catchment area (Fig. 4). In August, both DIN and SRP significantly depended on catchment area: positive for DIN and negative for SRP. Therefore, the improvement of the correlation between the DIN:SRP ratio and catchment size in August respective to July (see results above) depended mostly on the dynamics of SRP; the DIN:SRP ratio increased with catchment area, indicating a higher probability of P limitation in the lakes with larger catchments (Fig. 4).

The ratio of lake surface area (*S*) to catchment area (*A*) and lake volume (*V*) can be used in an index (*S/AV*) of the relative importance of atmospheric inputs in relation to runoff inputs. High *S/AV* values suggest a higher relative importance of atmospheric inputs. Otherwise, low *S/AV* values suggest a higher relative importance of runoff inputs. In Fig. 5, we show that the SRP increases were particularly evident in the lakes having a higher *S/AV* value.

The results obtained suggest that lakes in the Sierra Nevada with larger catchment areas are more likely to be P limited because of greater runoff inputs. The Chl *a* dependence on TP and (or) TN was assessed with a ridge regression (see Materials and methods), which considered TP and TN collinearity. For λ values between 0 and 0.1, β coefficients for log(TN) were not significant, whereas β coefficients for log(TP) were always significant ($p < 0.05$, at least). This result indicates that only TP had a significant effect on Chl *a* for the whole set of lakes. Nevertheless, catchment size determined the strength of correlation between Chl *a* and TP. Figure 6 shows the regression analysis between Chl *a* and TP for the lakes (Fig. 6A) and the changes in the explained variance of Chl *a* when the analysis was repeated sequentially in two ways. In the first analysis group (Figs. 6B and 6C, solid circles), we began calculating the regression for the four lakes with the smallest catchment areas. Then, we repeated the calculations including the lake with the next smallest catchment area, and so on. In the second analysis group (Figs. 6B and 6C, squares), the procedure was similar but starting with the four lakes with the largest catchment areas and adding sequentially the lake with the next largest

© 1999 NRC Canada

Fig. 2. Relationship between epilimnetic TN and TP concentrations in the Sierra Nevada study lakes during the two periods studied. The solid lines show examples of selected N:P mass ratios (by weight). The broken line shows the average TN:TP trend in world lakes found by Downing and McCauley (1992).

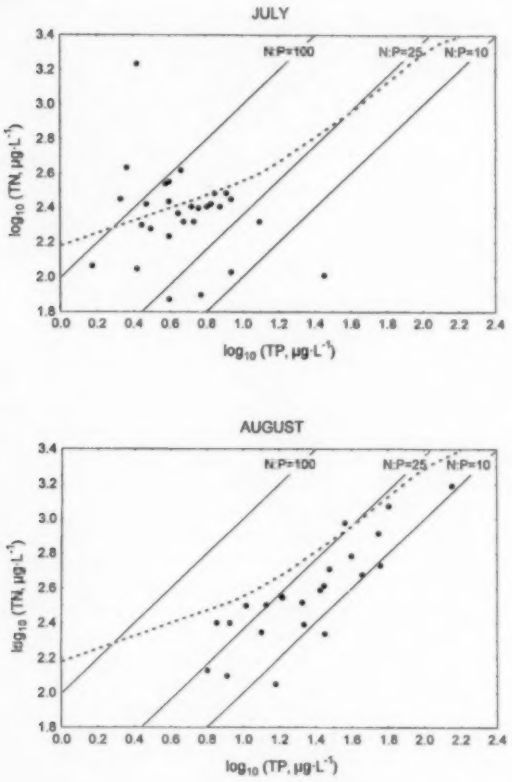
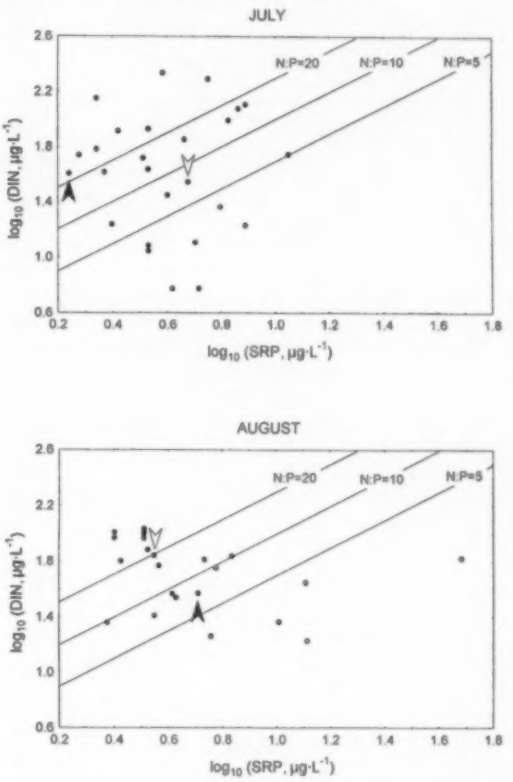


Fig. 3. Relationship between the epilimnetic DIN and SRP concentrations in the Sierra Nevada study lakes during the two periods studied. The solid lines show examples of selected N:P mass ratios (by weight). The lakes marked with arrowheads are the only ones that changed their ratios so greatly that they exceeded a value of 10 from July to August.



catchment area. Regressions were not significant when only the lakes with the smallest catchment areas were considered. With the inclusion of the ninth lake, the regressions started to be significant and the variance in Chl *a* explained by TP rose as lakes with successively larger catchments were included in the analysis. On the contrary, variance was best explained when only the lakes with larger catchment areas were included. Both regression sets suggest that lakes with small catchment areas are not P limited and this limitation is more likely as catchment area increases.

Discussion

The observed TN:TP reduction between July and August can be attributed to the increase in TP in lake waters. In July, TP was mostly in the form of SRP; however, DIN represented only a minor fraction of the TN. Levine and Schindler (1989) found that dissolved organic N has greater persistence than dissolved organic P in the water. They later used this to account for the fact that TN:TP ratios in fertilized enclosures were consistently higher than dissolved nutrient input ratios (Levine and Schindler 1992). According to our data, a higher persistence of dissolved organic N may explain the relatively high TN values in relation to TP in Sierra Nevada lakes in July. The small differences between SRP and TP suggest that P was not a limiting nutrient during this period.

The observed fall in TN:TP ratios from July to August can be understood as an accumulation of N and P in the plankton. Two pieces of evidence support this argument: first, Chl *a* increased between periods and second, TN and TP values increased in a proportion of 9:1 (by weight), which corresponds to plankton stoichiometry (Downing and McCauley 1992). Since TN increased in August, processes such as desnitrification or losses of N to the sediments are difficult to argue as alternative explanations.

In August, TN:TP ratios in the lakes were below world averages obtained by Downing and McCauley (1992). Low TN:TP ratios in Sierra Nevada fit the prediction of Downing and McCauley (1992) for lakes with small catchments in nonfertilized lands, suggesting that N:P ratios in precipitation were lower than in runoff in this area.

The importance and bioavailability of atmospheric P inputs for lakes with small catchments was demonstrated by Peters (1977). Recent research has shown that in those lakes, atmospheric precipitation can be a greater source of P than

© 1999 NRC Canada

Table 2. Results of ANCOVA indicating the effect of Sierra Nevada study lake outlets on $\log_{10}$(DIN:SRP) in August.

Source of variation	df	SS	*F*	*p*
Output	1	0.533	5.897	0.026
Both covariates	2	1.026	5.670	0.012
$\log_{10}$(area) covariate	1	0.735	8.131	0.011
$\log_{10}$(depth) covariate	1	0.495	5.471	0.031
Error	18	1.628		
Interaction				0.674

Note: Lakes were divided in two groups according to the presence or absence of outlets. Differences in mean $\log_{10}$(DIN:SRP) between groups (output) were tested including catchment area ($\log_{10}$(area)) and maximum depth ($\log_{10}$(depth)) as covariates. We show the effect of both covariates together and the single effect of each one. Nonsignificant differences between the slopes of the regression lines of covariate variables are given by the interaction probability value.

Fig. 4. Relationship between DIN (circles) and SRP (diamonds) concentrations and catchment area in the Sierra Nevada study lakes during the two periods studied. The broken vertical lines in August show reference DIN:SRP ratios for different catchment areas according to the regression obtained between DIN:SRP and catchment area in August (see text). July regression lines: $\log_{10}(DIN) = 1.100 + 0.384 \log_{10}(area)$ ($r^2 = 0.14$, $p < 0.05$); $\log_{10}(SRP) = 0.628 - 0.005 \log_{10}(area)$ (not significant). August regression lines: $\log_{10}(DIN) = 1.271 + 0.311 \log_{10}(area)$ ($r^2 = 0.28$, $p < 0.01$); $\log_{10}(SRP) = 1.101 - 0.305 \log_{10}(area)$ ($r^2 = 0.14$, $p < 0.05$).

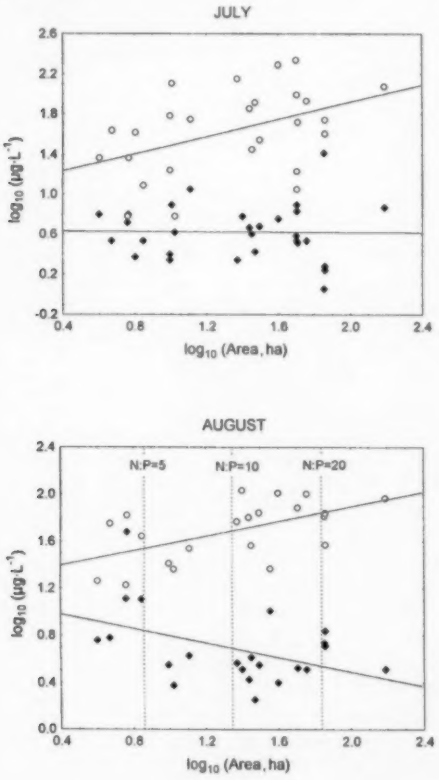
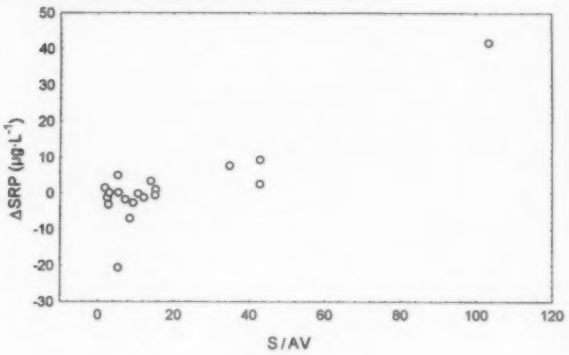


Fig. 5. Relationship between the increase in SRP (ΔSRP) from July to August in the Sierra Nevada study lakes and *S*/*AV*, where *S* is lake surface area (hectares), A is catchment area (hectares), and *V* is lake volume (cubic hectometres).



land runoff (Campbell 1994; Fee et al. 1994; Gibson et al. 1995). Especially low N:P ratios in the atmospheric precipitation of the Sierra Nevada may be expected as a consequence of the aforementioned Saharan dust. Talbot et al. (1986) measured in situ the chemical characteristics of the aerosol in an extensive Saharan dust plume. The main stream of the dust transport appeared at about 2500 m above sea level, showing a PO_4 concentration in the air of 265 ng·m^{-3} versus an NO_3 plus NH_4 concentration of 877 ng·m^{-3}, yielding an N:P ratio of 2.8 (by weight) for these compounds.

Low N:P ratios in the atmospheric inputs to the Sierra Nevada are also suggested by the positive correlation found between catchment area and the DIN:SRP ratio. This correlation indicates that lakes with smaller catchments could have more relative availability of P. Although both DIN and SRP may not reflect the exact availabilities of N or P for phytoplankton (Rigler 1966; Peters 1977), the DIN:SRP ratio, as a ratio of nutrient supply, has been used as an indicator of N or P limitation (Morris and Lewis 1988). The fact that most of the lakes studied here maintained DIN:SRP ratios during the two periods without exceeding the threshold value of 10 suggests that the observed increase in the planktonic biomass occurred under the same conditions of nutrient limitation for each lake. It has been argued that algal populations in small lakes are especially nutrient deficient because such lakes are well lit and have low turbulence (Fee et al. 1992). This argument can be applied to the Sierra Nevada lakes. Their maintenace of DIN:SRP ratios during phytoplankton growth may indicate the greater ability of dissolved inorganic compounds, compared with total compounds, to reflect external inputs. Therefore, the catchment size was related to the DIN:SRP ratio but not to the TN:TP ratio. Given the evidence, stoichiometrically supported, that nutrient recycling by herbivorous zooplankton accentuates the existing limiting conditions (Sterner 1990; Elser et al. 1995; Urabe et al. 1995), the more developed planktonic community in August could explain the improved relationship between catchment size and the DIN:SRP ratio observed in this month (Fig. 4). At this time, the failure of TP to explain Chl *a* variability when lakes with small catchments are considered agrees with a lack of P limitation in

© 1999 NRC Canada

Fig. 6. (A) Relationship between Chl *a* and TP in August in all the Sierra Nevada study lakes, (B) changes in the explained variance in Chl *a* by TP as lakes with increasing (solid circles) or decreasing (squares) catchment area are sequentially included in the regression (see text), and (C) the same as Fig. 6B but excluding the lake marked with an arrowhead in Fig. 6A. ns, regression not significant; *regression significant with $p < 0.05$; points not marked significant with $p < 0.01$ or higher significance.

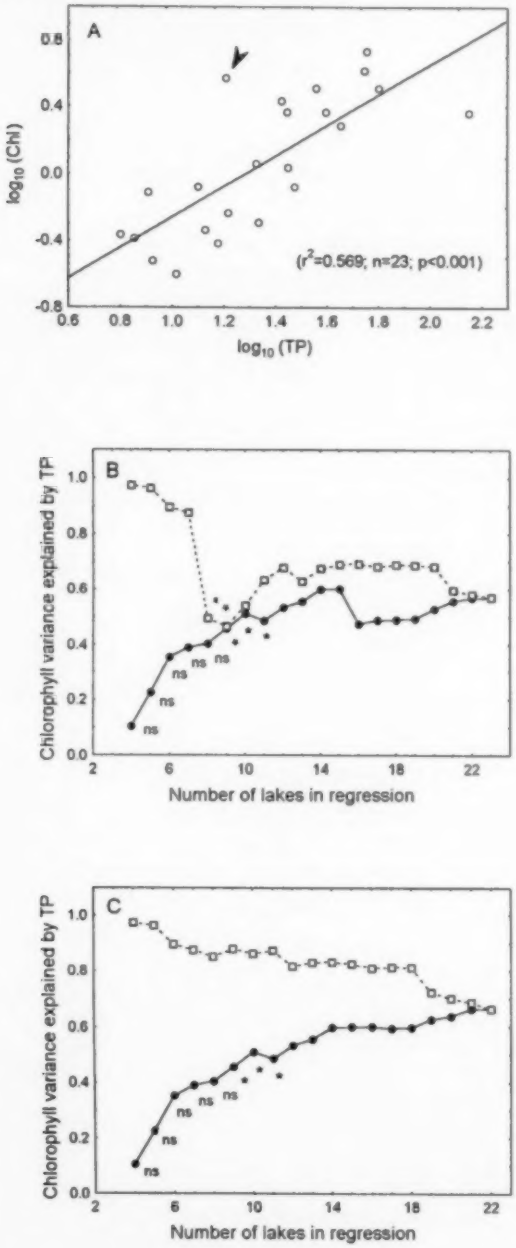
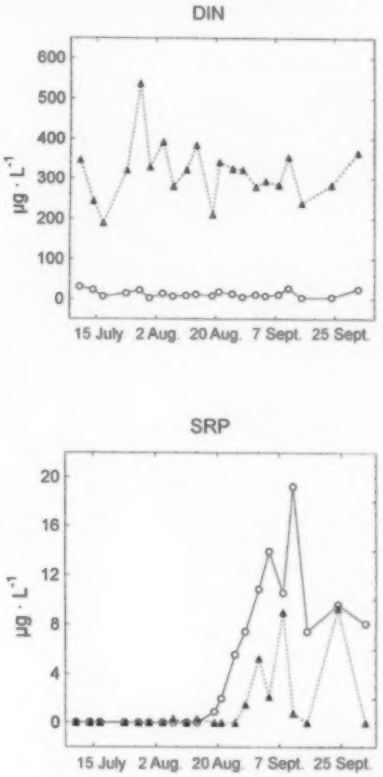


Fig. 7. Simultaneous dynamics of DIN and SRP during the entire ice-free period of 1986 in two Sierra Nevada lakes with different catchment areas, La Caldera (triangles) and Rio Seco (circles) (see Table 1) (data from Carrillo 1989).



these lakes, as the TP – Chl *a* relationship is especially well established in P-limited lakes (Dillon et al. 1988). Furthermore, in agreement with a predominant atmospheric origin of external P supplies to Sierra Nevada lakes, we found the highest SRP accumulation in the lake with the highest surface area relative to its catchment area and lake volume (Fig. 5).

A previous study describing detailed dynamics of nutrients in two lakes with different catchment areas (Carrillo 1989) (Fig. 7) also supports the abovementioned effect of catchment size on DIN:SRP ratios. The lake with higher catchment area (La Caldera) always showed higher DIN concentrations than the lake with smaller catchment area (Rio Seco) during the whole ice-free period. In both lakes, SRP increased during the second half of the season, but more conspicuously in the lake with the smallest catchment area (Rio Seco). Moreover, an increase in Chl *a* of about 100% was detected in La Caldera 5 days after one rain episode carrying Saharan dust (Carrillo et al. 1990).

On the other hand, this study provides evidence that TN:TP ratios of high mountain lakes within one geographical area change markedly at different times of the growing period. This result highlights the importance of performing comparative studies among lakes at the same seasonal stage. It also draws attention to the possibility of not finding the

© 1999 NRC Canada

expected relationships concerning TN:TP ratios when the stages for each lake are not comparable. On the other hand, the validity of the results obtained in this study should be tested for larger lakes.

Finally, our results show that the high TN:TP ratios found in high mountain lakes in central Europe (Kopácek et al. 1995) do not constitute a general pattern for the entire Northern Hemisphere. Although there is evidence of dramatic increases in N precipitation in eastern North America and western Europe during this century (Brimblecombe and Stedman 1982), there is also evidence that N precipitation is not uniform in all parts of the Northern Hemisphere (Lohr et al. 1989). Moreover, local factors, such as we have discussed for the Sierra Nevada, may substantially alter N:P ratios in atmospheric precipitation. With Saharan dust flux values in the western Mediterranean of about 14 tonnes·km^{-2}·year^{-1} (Loÿe-Pilot et al. 1986), this possibility is worthy exploring in the future.

Aknowledgements

We thank M. Villar-Argaiz and I. Moreno-Garrido for field and laboratory help. The manuscript was improved by reviewer comments and criticisms. This research was supported by CICYT projects NAT89/1111 and AMB94/0021.

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. (APHA). 1989. Standard methods for the examination of water and wastewater. 17th ed. APHA, New York.

Brimblecombe, P., and Stedman, D.H. 1982. Historical evidence for a dramatic increase in the nitrate component of acid rain. Nature (Lond.), **298**: 460–462.

Campbell, P. 1994. Phosphorus budgets and stoichiometry during the open-water season in two unmanipulated lakes in the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. **51**: 2739–2755.

Carrillo, P. 1989. Análisis de las interacciones tróficas en el plancton de un sistema oligotrófico. Ph.D. thesis, Universidad de Granada, Granada, Spain.

Carrillo, P., Cruz-Pizarro, L., and Morales-Baquero, R. 1990. Effects of unpredictable atmospheric allochthonous input on the light climate of an oligotrophic lake. Verh. Int. Ver. Limnol. **24**: 97–101.

Cruz-Pizarro, L. 1983. Reproductive activity of *Mixodiaptomus laciniatus* (Copepoda, Calanoida) in the high mountain Lake La Caldera (Spain). Hydrobiologia, **107**: 97–105.

Dillon, P.J., Nicholls, K.H., Locke, B.A., de Grosbois, E., and Yan, N.D. 1988. Posphorus–phytoplankton relationships in nutrient-poor soft-water lakes in Canada. Verh. Int. Ver. Limnol. **23**: 258–264.

Dillon, P.J., Molot, L.A., and Scheider, W.A. 1991. Phosphorus and nitrogen export from forested stream catchments in central Ontario. J. Environ. Qual. 1991. **20**: 857–864.

Downing, J.A., and McCauley, E. 1992. The nitrogen:phosphorus relationship in lakes. Limnol. Oceanogr. **37**: 936–945.

Elser, J.J., Lubnow, F.S., Marzolf, E.R., Bret, M.T., Dion, G., and Goldman, C.R. 1995. Factors associated with interannual and intrannual variation in nutrient limitation of phytoplankton growth in Castle Lake, California. Can. J. Fish. Aquat. Sci. **52**: 93–104.

Fee, E.J., Shearer, J.A., DeRuyn, E.R., and Schindler, E.U. 1992. Effects of lake size on phytoplankton photosynthesis. Can. J. Fish. Aquat. Sci. **49**: 2445–2459.

Fee, E.J., Hecky, R.E., Regehr, G.W., Hendzel, L.L., and Wilkinson, P. 1994. Effects of lake size on nutrient availability in the mixed layer during summer stratification. Can. J. Fish. Aquat. Sci. **51**: 2756–2768.

Gibson, C.E., Wu, Y., and Pinkerton, D. 1995. Substance budgets of an upland catchment. The significance of atmospheric phosphorus inputs. Freshwater Biol. **33**: 385–392.

Henriksen, A., and Brakke, D.F. 1988. Increasing contributions of nitrogen to the acidity of surface waters in Norway. Water Air Soil Pollut. **42**: 183–201.

Hoerl, A.E. 1962. Application of ridge analysis to regression problems. Chem. Eng. Prog. **58**: 54–59.

Kopácek, J., Procházková, L., Stuchlík, E., and Blazka, P. 1995. The nitrogen–phosphorus relationship in mountain lakes: influence of atmospheric input, watershed, and pH. Limnol. Oceanogr. **40**: 930–937.

Levine, S.N., and Schindler, D.W. 1989. Phosphorus, nitrogen, and carbon dynamics during the recovery of a lake from eutrophication. Can. J. Fish. Aquat. Sci. **46**: 2–9.

Levine, S.N., and Schindler, D.W. 1992. Modification of the N:P ratio in lakes by in situ processes. Limnol. Oceanogr. **37**: 917–935.

Lohr, R.C., Ryding, S.O., and Sonzogni, W.C. 1989. Nutrient loading estimation in a water body. *In* The control of eutrophication of lakes and reservoirs. *Edited by* S.O. Ryding and W. Rast. UNESCO, Paris.

Loÿe-Pilot, M.D., Martin, J.M., and Morelli, J. 1986. Influence of Saharan dust on the rain acidity and atmospheric input to the Mediterranean. Nature (Lond.), **321**: 427–428.

McCauley, E., Downing, J.A., and Watson, S. 1989. Sigmoid relationships between nutrients and chlorophyll among lakes. Can. J. Fish. Aquat. Sci. **46**: 1171–1175.

Molot, L.A., and Dillon, P.J. 1991. Nitrogen/phosphorus ratios and the prediction of chlorophyll in phosphorus-limited lakes in central Ontario. Can. J. Fish. Aquat. Sci. **48**: 140–145.

Morales-Baquero, R., Cruz-Pizarro, L., and Carrillo, P. 1989. Patterns in the composition of the rotifers communities from high mountain lakes and ponds in Sierra Nevada (Spain). Hydrobiologia, **186/187**: 215–221.

Morris, D.P., and Lewis, W.M. 1988. Phytoplankton nutrient limitation in Colorado mountain lakes. Freshwater Biol. **20**: 315–327.

Moulin, C., Lambert, C.E., Dulac, F., and Dayan, U. 1997. Control of atmospheric export of dust from North Africa by the North Atlantic Oscillation. Nature (Lond.), **387**: 691–694.

Murphy, J., and Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Ann. Chim. Acta, **27**: 31–36.

Peters, R.H. 1977. The availability of atmospheric orthophosphate. J. Fish. Res. Board Can. **34**: 918–924.

Rigler, F.H. 1966. Radiobiological analysis of inorganic phosphorus in lake water. Verh. Int. Ver. Limnol. **16**: 465–470.

Rigler, F.H., and Peters, R.H. 1995. Science and limnology. *In* Excellence in ecology. Vol. 6. *Edited by* O. Kinne. Ecology Institute, Oldendorf/Luhe, Germany.

Rodier, J. 1990. Análisis de las aguas. Omega, Barcelona, Spain.

Sánchez-Castillo, P., Cruz-Pizarro, L., and Carrillo, P. 1989. Caracterización del fitoplancton de las lagunas de alta montaña de Sierra Nevada (Granada, España) en relación con las características fisico-químicas del medio. Limnetica, **5**: 37–50.

Sterner, R.W. 1990. The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. Am. Nat. **136**: 209–229.

© 1999 NRC Canada

Strickland, J.D.H., and Parsons, T.R. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. No. 167.

Talbot, R.W., Harris, R.C., Browell, E.V., Gregory, G.L., Sebacher, D.I., and Beck, S.M. 1986. Distribution and geochemistry of aerosols in the tropical North Atlantic troposphere: relationships to Saharan dust. J. Geophys. Res. **91**(D4): 5173–5182.

Urabe, J., Nakanishi, M., and Kawabata, K. 1995. Contribution of metazoan plankton to the cycling of nitrogen and phosphorus in Lake Biwa. Limnol. Oceanogr. **40**: 232–241.

© 1999 NRC Canada

Growth, condition, and environmental relationships in Atlantic cod (*Gadus morhua*) in the northern Gulf of St. Lawrence and implications for management strategies in the Northwest Atlantic

J.-D. Dutil, M. Castonguay, D. Gilbert, and D. Gascon

Abstract: Northern Gulf of St. Lawrence Atlantic cod (*Gadus morhua*) is one of several stocks that collapsed in eastern Canada following a long period of intensive exploitation. Surplus and net production per capita became nil or negative in the mid-1980s so that any level of exploitation would have caused a decline of the stock. This was partly explained by a marked decline in growth production and is consistent with smaller sizes-at-age but also lower condition factor values during the same period. Correlations between size-at-age and temperature were not significant when corrected for autocorrelation, but slopes were always positive, suggesting higher growth rates at higher temperatures. Smaller sizes-at-age in the 1980s were not associated with changes in the fishery or increased fishing mortality, nor were they consistent with the density-dependence hypothesis. Lengths at age 8 decreased by more than 10 cm as the stock decreased 10-fold in abundance. While size-at-age and temperature covary in cod when all stocks are examined, size–temperature relationships are not as clear if the analysis is restricted to cold-water stocks, possibly because of differences in food availability. Biological production varies from year to year and among stocks and should be taken into consideration when managing fisheries in variable or extreme environments.

Résumé : Comme d'autres stocks dans l'est du Canada, le stock de morue franche (*Gadus morhua*) du nord du Golfe du Saint-Laurent s'est effondré après une longue période de pêche intensive. La production individuelle excédentaire a chuté au milieu des années 1980 de sorte que toute exploitation ne pouvait se solder que par une production nette négative. Ceci s'explique en partie par un ralentissement de la croissance et n'est pas étranger au fait que la taille à l'âge et l'indice de condition ont chuté pendant cette période. Les corrélations entre la taille à l'âge et la température du Golfe du Saint-Laurent ne sont pas significatives lorsque corrigées pour l'autocorrélation, mais la pente de ces relations est toujours positive ce qui suggère que la croissance est meilleure lorsque la température augmente. La diminution des tailles à l'âge ne semble pas s'expliquer par un taux de mortalité par pêche plus élevé ni par des changements dans la sélectivité des engins de pêche. Elle ne résulte pas non plus d'une augmentation des densités puisqu'une baisse de 10 cm de la longueur à 8 ans s'est produite alors que le stock est devenu 10 fois moins abondant. La longueur à l'âge varie selon la température lorsqu'on considère l'ensemble des stocks de l'Atlantique, mais cette relation est floue lorsque l'analyse porte uniquement sur les stocks d'eau froide, dont la croissance pourrait refléter davantage des différences dans l'abondance de nourriture. Puisqu'elle varie d'année en année et d'un stock à l'autre, la production biologique est un élément à intégrer au processus de gestion si l'on veut assurer la pérennité des stocks vivant dans un environnement variable ou extrême.

Introduction

Atlantic cod (*Gadus morhua*) are widely distributed in the North Atlantic. Growth rate varies among stocks and may vary from year to year within stocks. These stocks all sustain intensive fisheries and many from the Northwest Atlantic have collapsed in the early 1990s. Fishing has a major impact on the age and size distribution of fish in exploited populations, but environmental conditions, which vary across the range of the species, may also explain marked differences in size-at-age among stocks. Brander (1995) compared mass-at-age in the landings for 17 cod stocks. Mass-at-age varied considerably, from 0.6 kg for a 4-year-old cod in the northern and southern Gulf of St. Lawrence up to 7.3 kg for a 4-year-old cod in the United Kingdom. Variability in size was explained mainly by two factors, age and temperature. Mass of 4-year-old cod increases on average 30% among stocks for every 1°C increase in ambient temperature (Brander 1995). Lengths also vary considerably among stocks in the Northwest Atlantic, and again, temperature is a significant covariate in the length–age relationships.

Received February 12, 1999. Accepted July 29, 1999.
J15020

J.-D. Dutil,[1] M. Castonguay, D. Gilbert, and D. Gascon. Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, QC G5H 3Z4, Canada.

[1]Author to whom all correspondence should be addressed. e-mail: dutiljd@dfo-mpo.gc.ca

© 1999 NRC Canada

Furthermore, declining sizes-at-age of cod on the Scotian Shelf were at least partly accounted for by changes in water temperatures (Campana et al. 1995).

Determinants of growth rate have been well studied in cod under laboratory conditions. Temperature controls growth rate. Near 0°C, food consumption (Waiwood et al. 1991) and rate of gastric evacuation (Jobling 1982) are considerably reduced. With increasing temperature, growth rate increases to a maximum around 14°C (Jobling 1988). The combined effects of ration (e.g., Hawkins et al. 1985), fish size, and temperature have been described by Jobling (1988). These factors and others interact in the wild and result in variations in growth rate among individuals. They may also play a role in the observed differences in size-at-age among stocks (Brander 1995; Campana et al. 1995) and from year to year within stocks.

Size-at-age varies from year to year in all stocks, but a downward trend in size-at-age was observed in the 1980s in many stocks from the Northwest Atlantic. While size-at-age does not appear to have changed in southern locations (western Scotian Shelf, Bay of Fundy, Georges Bank, and Gulf of Maine; Campana et al. 1995), marked declines have been reported for other stocks, including Labrador and northern Grand Bank (Lilly et al. 1998) and eastern Scotian Shelf (Campana et al. 1995). The two stocks in the Gulf of St. Lawrence show no exception, with decreases in mass-at-age of 40–50% relative to the 1970s (Hanson and Chouinard 1992; Chouinard and Fréchet 1994). Smaller sizes-at-age were accompanied by changes in individual condition in some stocks. Fulton's condition index K, a reliable indicator of the energetic condition in cod (Lambert and Dutil 1997*a*), declined in the late 1980s and early 1990s in the northern Gulf of St. Lawrence (Lambert and Dutil 1997*b*) and was low, declining, or unchanged in the late 1980s in other areas (Taggart et al. 1994; Dutil et al. 1995; Lilly 1997).

Smaller sizes-at-age may not reflect actual changes in growth rate. Growth rate is calculated from the size-at-age of survivors. Intensive fisheries may modify the age and size structure of a stock by selectively removing ages and sizes fully vulnerable to gears and by removing fast-growing younger fish and slow-growing older fish among ages not fully vulnerable to gears. This results in slower or faster apparent growth rates (Hanson and Chouinard 1992; Swain and Wade 1993; Hutchings 1996). Changes in fishing mortality and (or) gear selectivity may impose a temporal trend in sizes-at-age. Slower growth rates may also result from changes in the genetic makeup of the stock through long-term selection of slow- or fast-growing fish exerted by an intensive fishery (Sheridan 1995).

Smaller sizes-at-age and lower condition could have resulted from changes in environmental conditions as well, particularly lower water temperatures through direct effects on metabolic rates and scope for growth and (or) through indirect effects on distribution and prey availability. The Gulf of St. Lawrence has been experiencing below-normal water temperatures in the cold intermediate layer (CIL) since the mid-1980s (Gilbert and Pettigrew 1997). Record low temperatures were also observed over the Newfoundland Shelf in the early 1990s (Colbourne et al. 1997). Myers and Cadigan (1995) examined sequential population analysis (SPA) data for northern cod in order to determine whether its collapse could be attributed to a sudden increase in natural mortality. The exact period for this discrete event, viewed as a cold spell, was the first half of 1991, as the loss of 500 000 t of fish could not be accounted for in the 1992 stock assessment (Lear and Parsons 1993). They concluded that northern cod had collapsed as a result of overfishing and that environmental conditions had had little or no impact relative to fishing mortality (also see Hutchings and Myers 1994; Hutchings 1996). Natural mortality rates were high in the 1990s in the northern Gulf of St. Lawrence (Dutil and Lambert 2000), but while extremely poor environmental conditions could have suddenly increased natural mortality, slowly deteriorating environmental conditions, due to changes in physical factors and (or) food availability, should have affected individual growth rates and condition first and survival only second.

This study explores relationships between growth, condition, and environmental temperature among cod stocks in the North Atlantic. The case of the northern Gulf of St. Lawrence cod stock, which lives in cold water and was characterized by declining individual size and condition prior to its collapse in the late 1980s and early 1990s, is examined in more detail. Long-term trends in mean size-at-age of cod in the fishery and research surveys were critically analyzed to determine whether they were correlated with variations of water temperature in the northern Gulf of St. Lawrence. Based on these results, we examine the role that environmental limitations may have played in the collapse of this stock and several others under high fishing pressure in the early 1990s. We also discuss the impact of our findings on current management strategies for cod stocks in the Northwest Atlantic.

Materials and methods

Size-at-age

Lengths and weights were drawn from assessment documents. Mean weights-at-age in assessment documents are calculated from length and age data of fish in landings and from length–weight relationships obtained in research surveys (Gavaris and Gavaris 1983). Basically, landings are sampled in ports and stratified by fishing area, fishing gear, and time. For each landing sampled, a length frequency is tallied on a random subsample. Each stratified length sample is weighted by its associated landing, yielding the total number of fish caught at length by the fishery. Length-classes are subsampled for otoliths so as to obtain unbiased samples of ages-at-length. From this, an age–length key is prepared that then yields the catch-at-age. Research surveys provide individual length and weight data from which length–weight relationships are calculated. Weights-at-age of landings are then obtained by multiplying the age–length key matrix (commercial samples) and the mean weight-at-length vector (research survey samples). Some assessments use a new length–weight relationship every year and some do not.

Research surveys in the northern Gulf of St. Lawrence were conducted in January 1978–1994 (no survey in 1982) (MV *Gadus Atlantica*). Surveys were also conducted in July 1984 and in August 1985–1997 (MV *Lady Hammond*, 1984–1989; RV *Alfred Needler*, 1990–1997). Individual lengths are available for each year except 1984 and individual weights are available for January (1983–1994) and August (1988–1997). Lengths-at-age were weighted by the catch (Morgan and Hoenig 1997). Bottom trawls used in the research surveys had a stretched mesh of 40 mm with a

© 1999 NRC Canada

codend liner of 30 mm (winter), 32 mm (summer, 1984–1989), or 19 mm (summer, 1990–1997). Survey methods and fishing gears are described in Castonguay et al. (1999).

Size-at-age indices (1957–1997) were calculated from standardized lengths, i.e., lengths from which we subtracted the mean and divided by the standard deviation. Length data were standardized within each age group (4, 6, and 8) using the mean and standard deviation of three time series: landings, winter research surveys (1978–1994), and summer (1984–1997) research surveys. Few data were available for landings prior to 1974 (May 1968, fig. 8, 1962–1965; Pinhorn 1969, figs. 11A–11F, 1957–1965). These data are much more limited in spatial and temporal coverage than data collected in 1974–1993 (Fréchet et al. 1994). The fishery has been closed since 1994 except for small-scale reopenings since 1997. Figures in May (1968) and Pinhorn (1969) were scanned (ScanJet 4c/t, Hewlett-Packard) and lengths were estimated using image analysis software (Sigma Scan-Pro). For each series, we averaged the normal deviates for the three age groups in each year and used this value as an index of length for cod having recruited to the fishery. Length data for 4-year-old cod in landings were excluded because earlier analyses had shown that they were biased due to selective fishing of large-sized individuals.

Temperature

Temperature indices were calculated using data between May 1 and September 30 of each year in the Atlantic Fisheries Adjustment Program database of salinity and temperature at the Bedford Institute of Oceanography (Dartmouth, N.S.). Gilbert et al. (1997) published Gulf-wide area-weighted mean temperatures from 1985 to 1995 for three layers: 30–100, 100–200, and 200–300 m. We extend their time series back in time to 1946 by using their weightings for 18 subareas of the Gulf. In the 200–300 m layer, missing data in 1958, 1964, 1972, and 1981 were filled by linear interpolation using data from the preceding and following years. We adjusted our estimates of temperature in the 30–100 m layer for seasonal warming by assuming an average May–September warming rate of 0.0115°C/day (Petrie et al. 1996, p. 250) and extrapolating all temperatures to a common midsummer date, July 15.

Variations in size-at-age were compared with changes in CIL temperature. The CIL is a layer of cold water (between −1.8 and +3.0°C) that typically occurs in summer between 30 and 125 m depth in the Gulf of St. Lawrence. Bottle measurements with reversing thermometers were excluded from our CIL core temperature calculations, as in Gilbert and Pettigrew (1997). Temperatures for the CIL in the Gulf of St. Lawrence were expressed as deviations from the 1948–1994 mean core temperature (Gilbert and Pettigrew 1997).

Temperature–size relationships were tested using linear regression and cross-spectrum analysis (Jenkins and Watts 1968). Length data were standardized (see above), but each series (4-, 6-, 8-year-olds) was analyzed individually. New temperature indices representative of the entire life span of 4-, 6-, and 8-year-old cod were generated for each layer (core of the CIL and 30–100, 100–200, and 200–300 m). For example, for 4-year-old fish in 1980, the life span CIL index was calculated as the average of the annual deviations from the 1948–1994 mean core temperature from 1977, when the cohort was 1 year old and had settled on the bottom, to 1980, when the cohort was 4 years old and lengths were measured. Cross-spectrum analyses were restricted to CIL index data.

Relationships between size and several other factors were examined as follows. Cumulative fishing mortality over a cohort life span was plotted against size-at-age using age-specific fishing mortality rates of each year-class from age 3 to age 5 and from age 3 to age 7 versus lengths at ages 6 and 8, respectively, in January (research surveys; data from Fréchet and Schwab 1998). We calculated a Pearson correlation coefficient between the median latitude of the geographic distribution of cod in January (see Castonguay et al. 1999) and standardized lengths and between stock biomass (fish older than 2 years, 1974–1997; Fréchet and Schwab 1998) and standardized lengths.

We accounted for autocorrelation in correlation and regression analyses by calculating an effective sample size (N^*). The N^* can be much smaller than the sample size N when the data are strongly autocorrelated (Pyper and Peterman 1998). The formula that we used to compute N^* is

$$(1) \qquad \frac{1}{N^*} = \frac{1}{N} + \frac{2}{N}\sum_{j=1}^{N/5} r_{XX}(j) r_{YY}(j)$$

where $r_{XX}(j)$ and $r_{YY}(j)$ are the autocorrelations of X and Y at lag j, defined here with the additional weighting factor proposed by Pyper and Peterman (1998, p. 2133):

$$(2) \qquad r_{XX}(j) = \frac{N}{N-j} \times \frac{\sum_{t=1}^{N-j}(X_t - \bar{X})(X_{t+j} - \bar{X})}{\sum_{t=1}^{N}(X_t - \bar{X})^2}$$

Length back-calculations

Back-calculations were done on otoliths using the Fraser–Lee procedure with the intercept of the fish size versus otolith size relationship estimated from a geometric mean regression, as recommended by Ricker (1992) for back-calculations based on scales. Smedstad and Holm (1996) compared six back-calculation procedures for Atlantic cod otoliths and rated Ricker's (1992) as one of the top two methods. A fork length (L, centimetres) versus otolith radius (O, micrometres) relationship was calculated with a geometric mean regression using 279 cod 22–90 cm long (3- to 8-year-olds) that had been caught during research surveys in January 1978, 1984, 1989, and 1994 (70 otoliths/year with one outlier discarded). When X and Y variables are divided by their respective standard deviations, the slope (v) of their geometric mean regression becomes 45°, i.e., v = 1 (Ricker 1973, 1984). The intercept (K) of the geometric mean regression is calculated as usual (Sokal and Rohlf 1995, p. 544):

$$(3) \qquad K = \bar{L} - \mathrm{v}\bar{O}.$$

The distance from the otolith center to annuli corresponding to ages 1–7 were measured using a Bioquant® image analysis system at 12× magnification on 8-year-old cod otoliths cut through the center and polished. Fish used here were randomly sampled from 8-year-old fish in research surveys conducted in January. Two years (1981 and 1984) were selected in the period when fish were larger and two in the period when fish were smaller (1989 and 1994) (20 otoliths/year). Lengths-at-age of individual fish were back-calculated using the Fraser–Lee method (Ricker 1992, eq. 2):

$$(4) \qquad L_i = ((L - K)\ O_i\ O^{-1}) + K$$

where L_i is the individual back-calculated length at age i, O_i is the annulus measurement at age i, and L, O, and K are as above. Back-calculated mean lengths-at-age were then compared by year-class with weighted mean lengths-at-age (from the winter research vessel survey) at the beginning of the year for exploited ages (4–7). Lengths of 8-year-old fish used in back-calculations were also shown for comparison with weighted lengths of 8-year-old fish from the research vessel survey.

Production analysis

Production was broken down into its various components following Rivard (1982), using the SPA for 1998 (Fréchet and Schwab 1998). The addition of biomass to the exploitable popula-

© 1999 NRC Canada

tion from the recruiting age-class, i.e., 3-year-old cod in the northern Gulf, was calculated as

$$(5) \quad R_t = N_{t,3}\, w_{t,3}$$

where R_t is recruitment, $N_{t,3}$ is the number of 3-year-old cod in year t in the stock, and $w_{t,3}$ is the mean weight of 3-year-old cod in year t. Losses occur through fishing mortality and natural mortality:

$$(6) \quad \mathrm{BF}_t = \sum_{a=3}^{n} C_{t,a} w_{t,a}$$

$$(7) \quad \mathrm{BM}_t = \sum_{a=3}^{n} ((N_{t,a} - N_{t+1,a+1}) - C_{t,a}) w_{t,a}$$

where BF_t is the biomass landed in year t, BM_t is the biomass lost from natural causes, $C_{t,a}$ is the number of fish landed at age a in year t, and $N_{t,a}$ is the number of fish at age a in year t in the stock. Net production (NP_t) and surplus production (SP_t) were calculated as

$$(8) \quad \mathrm{NP}_t = \left(\sum_{a=3}^{n} ((N_{t+1,a+1} w_{t+1,a+1}) - (N_{t,a} w_{t,a})) \right) + R_t$$

$$(9) \quad \mathrm{SP}_t = \mathrm{NP}_t + C_t$$

and somatic growth (G_t) was estimated as

$$(10) \quad G_t = \sum_{a=3}^{n} N_{t+1,a+1} (w_{t+1,a+1} - w_{t,a}).$$

Equations 5–9 were divided by stock size (3 years and older) at the beginning of year t to yield production estimates on a per capita basis, but eq. 10 was divided by stock size at year $t + 1$ to exclude a mortality component from the calculation of G.

Growth rate and condition

Annual growth rates were estimated from weights-at-age in landings (Baird et al. 1992*a*, 1992*b*; Bishop and Murphy 1992; Campana and Hamel 1992; Fréchet and Schwab 1992; Hunt and Buzeta 1992; ICES 1992*a*, 1992*b*, 1992*c*, 1992*d*; Mohn and MacEachern 1992; Sinclair et al. 1995). We used data for 1990 (western English Channel (ENGC, ages 0–6), Bristol Channel and Celtic Sea (CELS, ages 1–6), North Sea (NORS, ages 1–7), and west Scotland (WSCD, ages 1–7)) and 1991 (Georges Bank (GSBK, ages 1–8), Faroe Plateau (FARP, ages 2–8), Labrador and Grand Bank (LGBK, ages 2–8), eastern and western Scotian Shelf (ESSF, WSSF, ages 2–8), southern Grand Bank (SGBK, ages 3–8), Saint-Pierre Bank (SNFD, ages 3–8), northern and southern Gulf of St. Lawrence (NGSL, SGSL, ages 3–8), northeast Arctic (NEAC, ages 3–8), east and west Greenland (EWGD, ages 3–8), Iceland (ICED, ages 3–8), and Irish Sea (IRHS, ages 1–6)). Age–weight plots were fitted with a third-degree polynomial from which we calculated the age of a 1-kg fish (predictor variable: weight). Values for GSBK and ICED were extrapolated. Weight–age plots were then fitted with a third-degree polynomial to assess the weight of cod 1 year later (W_2) (predictor variable: age). Values of R^2 for both fits were always greater than 0.98. The instantaneous mean daily growth rate (Ricker 1979) was calculated as

$$(11) \quad G = (\ln(W_2) - \ln(1.0))/365 = \ln(W_2)/365.$$

The maximal growth rate expected for a 1-kg fish was estimated using Jobling's (1988) relationship between G_{max}, size, and temperature. Stock-specific ambient temperatures reported by Brander (1995) were used to compute G_{max}.

Condition factor – size relationships were examined using lengths and weights at age 6 in both landings and research surveys. Few data were available: (*i*) LGBK, SNFD, Fleming (1960), his tables 2 and 3 for various areas around Newfoundland, summer 1947–1950, with gutted gilled weights transformed to round weights following May (1968); (*ii*) NGSL, January (1979–1981 and 1983–1994) and August (1987–1997) research surveys; (*iii*) SGSL, September (1972–1985 and 1987–1994) research surveys, weights from table 11 in Sinclair et al. (1996) and lengths from length–weight relationships in the assessment documents; (*iv*) NEAC, U.S.S.R., November–December (1984–1990) research surveys (ICES 1992*b*); (*v*) IRHS, landings 1982–1994, CELS, landings 1984–1988, and ENGC, landings 1988 and 1991–1996 (M.J. Boon, Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, U.K., personal communication); (*vi*) GSBK (5Z), landings 1979–1991, from table 7 in Hunt and Buzeta (1992). The condition factor was calculated as

$$(12) \quad K = 100 \times \text{total weight (g)} / \text{length (cm)}^3.$$

Results

Lengths-at-age of cod in the northern Gulf of St. Lawrence varied among years. Lengths of 4-year-old cod showed no clear trend in the period 1974–1994, but 6- and 8-year-old cod were smaller in 1985–1994 than in the earlier period (Fig. 1). Commercial samples provide the longest time series (1974–1994) and show no change in the size of 4-year-olds, a stepwise decrease in the size of 6-year-olds in 1983–1985, and a more gradual decrease in the size of 8-year-olds during the 1980s. Winter and summer research surveys show similar trends, with 6- and 8-year-old cod in landings and research surveys showing similar size. In contrast, 4-year-old cod were larger in landings than in the surveys.

Lengths-at-age in the period 1974–1994 were within the range of historic values for this stock. Early records date back to the 1940s. For example, lengths of 6-year-old cod ranged from 48.7 to 56.3 cm in early studies based on landings and research surveys (Thompson 1943; Fleming 1960; May 1968; Wiles and May 1968; Pinhorn 1969; Minet 1978). Similarly, lengths of 6-year-old cod in landings ranged from 47.8 to 56.8 cm in the period 1974–1994 (Fréchet et al. 1994) and from 45.5 to 55.8 cm and from 47.5 to 53.5 cm in winter and summer research surveys, respectively, conducted from 1978 to 1994.

Temperature varied in the three layers examined (30–100, 100–200, and 200–300 m) in the period 1946–1997 (Fig. 2). The early 1980s were exceptionally warm, positive anomalies being observed in the three layers. The early 1990s were unusually cold, with negative anomalies in the three layers. Low temperatures were observed earlier (late 1940s and late 1960s), but the high temperatures observed in the early 1980s were unprecedented. The composite index of temperature for these layers (30–300 m) shows a major decline by as much as 2°C over the bulk of the water column in the period 1980–1991 (Fig. 2).

Cross-spectrum analyses between length-at-age and the CIL index yielded no significant coherence ($P > 0.05$) (Thompson 1979) for 4-, 6-, and 8-year-old fish at the 10-year period, where most of the variance of the CIL time series is found. Similarly, correlations between length-at-age and four indices of temperature were not significant when accounting for autocorrelation ($P > 0.05$) (Table 1). Linear regressions explained a greater proportion of the variability in length-at-age when the CIL core or the 30–100 m indices

© 1999 NRC Canada

Fig. 1. Length of 4-, 6-, and 8-year-old Atlantic cod in the northern Gulf of St. Lawrence from 1974 to 1994. Commercial (open triangles) and research survey data for January (solid triangles) and August (solid circles) are shown. Data for landings and summer survey are shifted 6 and 8 months on the *x*-axis, respectively.

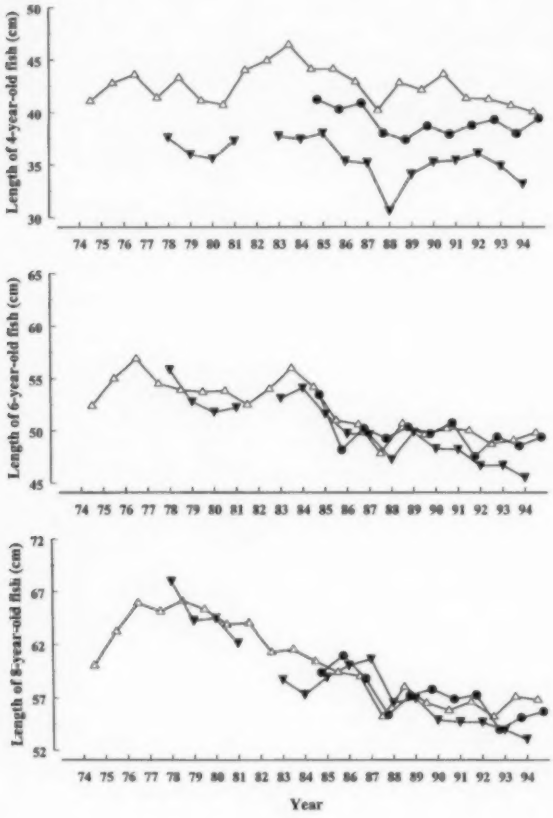
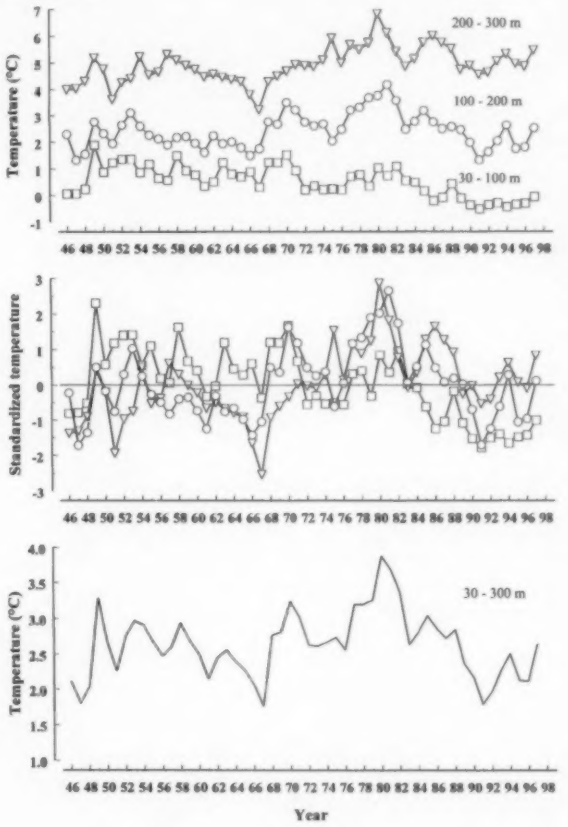


Fig. 2. Indices of temperature averaged over the entire Gulf of St. Lawrence for the 30–100 m (squares), 100–200 m (circles), and 200–300 m (triangles) layers. The top panel shows the actual temperatures in each layer, and the middle panel shows the standardized temperatures (zero mean, unit variance). The bottom panel shows the mean temperature of the three layers (30–300 m).



were used (Table 1) but a lesser proportion for the 100–200 m index and more particularly the 200–300 m index. Correcting for autocorrelation resulted in very low degrees of freedom (range 3–6).

The positive slopes of all 12 regressions (Table 1) suggest generally faster growth at higher temperatures, but no clear pattern emerged when size and CIL indices were plotted as a function of time. Smaller sizes in the mid-1960s and mid-1980s matched colder temperatures. In contrast, larger sizes in the late 1970s and late 1990s did not match warmer temperatures (Fig. 3). Fish caught in the 1981–1984 period were larger on average and had experienced warmer CIL temperatures over their life span, while fish caught in the period 1987–1996 and in particular in the period 1991–1994 were smaller and lived in a colder environment (Fig. 4). The years 1976 and 1997 appeared as outliers in the plots.

Length-at-age and median latitude of the northern Gulf cod's geographic distribution in January, as measured in the research survey, were positively correlated (Fig. 3). This correlation was significant even when the data were corrected for autocorrelation ($r = 0.90$, $n = 12$, effective $n = 5$, df = 3, $P < 0.05$). There was also a positive correlation between length-at-age and stock biomass (3 years and older), but this correlation was not significant when the data were corrected for autocorrelation ($r = 0.59$, $n = 24$, effective $n = 5$, df = 3, $P > 0.05$).

Smaller lengths of cod at ages 6 and 8 in the late 1980s in the northern Gulf of St. Lawrence were not associated with increased rates of fishing mortality. The cumulative rates of fishing mortality over the first 5 years of life (fishing mortality is near zero in cod younger than 4 years old) for year-classes 1972–1988 did not show any relationship with length of 6-year-old cod in January (Fig. 5). Cumulative fishing mortality in the first 5 years of life actually decreased slightly, while length-at-age decreased by as much as 9 cm between 1978 and 1990. From 1991, cumulative fishing mortality increased markedly (threefold), but length of 6-year-old cod decreased by less than 2 cm. Similarly, fishing mortality over the first 7 years of life for year-classes 1970–1986 did not increase in the period 1978–1990, while length of 8-year-old cod decreased by as much as 13 cm.

© 1999 NRC Canada

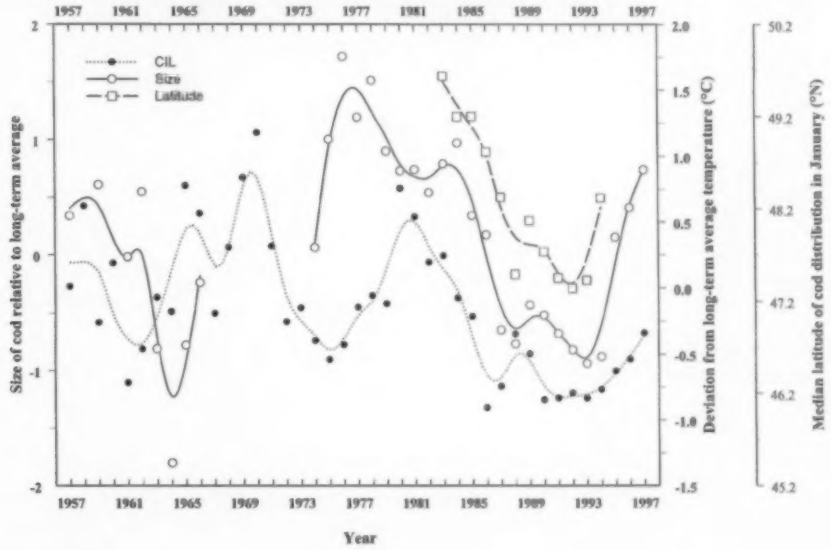
Table 1. Relationship between length-at-age and four temperature indices averaged over the fish's life span for 4-, 6-, and 8-year-old Atlantic cod in the northern Gulf of St. Lawrence (standardized data).

Climate index	Age group	Period	N	N^*	Slope	r^2	P
CIL core temperature	4[a]	1978–1997	20	8	1.09	0.25	0.207
CIL core temperature	6	1974–1997	24	6	1.18	0.25	0.308
CIL core temperature	8	1974–1997	24	5	1.38	0.28	0.355
30–100 m temperature	4[a]	1978–1997	20	8	0.73	0.13	0.375
30–100 m temperature	6	1974–1997	24	6	1.11	0.27	0.291
30–100 m temperature	8	1974–1997	24	5	1.30	0.32	0.320
100–200 m temperature	4[a]	1978–1997	20	8	0.43	0.09	0.460
100–200 m temperature	6	1974–1997	24	6	0.70	0.19	0.386
100–200 m temperature	8	1974–1997	24	5	0.78	0.17	0.489
200–300 m temperature	4[a]	1978–1997	20	8	0.24	0.01	0.800
200–300 m temperature	6	1974–1997	24	7	0.67	0.07	0.567
200–300 m temperature	8	1974–1997	24	6	0.18	0.00	0.897

Note: Results from linear regression analysis are shown, taking into account the effects of autocorrelation on the effective number of degrees of freedom ($N^* - 2$).

[a]Commercial data were excluded because earlier analyses had shown that lengths were biased due to selective fishing of large-sized 4-year-old cod. A missing value in 1982 was filled by linear interpolation.

Fig. 3. Size of 4-, 6-, and 8-year-old Atlantic cod (standardized data), median latitude of cod distribution in January, and deviations from the 1948–1994 mean temperature of the CIL in the Gulf of St. Lawrence for the period 1957–1997. Both commercial data (May 1968; Pinhorn 1969; Fréchet et al. 1994) and research survey data are used to describe variations in length-at-age. Symbols represent actual observations and lines were smoothed using locally weighted least-squares.



Back-calculated and measured lengths at age 4 and 5 differed, particularly for the year-class born in 1981 (Table 2). After body length (L) and otolith radius (O) were divided by their respective standard deviations, the geometric mean regression used in back-calculations was $L = O - 1.095$, with an adequate pattern of residuals and $r^2 = 0.865$ (Fig. 6). Except for the 1981 year-class, measured lengths of the back-calculated sample (age 8) were larger than measured lengths of the weighted sample from the January survey. Nevertheless, back-calculated lengths at age 4 and to a lesser extent at age 5 were always smaller than the estimated average length in the survey in corresponding years. Larger differences were observed at age 4 (1985), age 5 (1987), and to a lesser extent age 6 (1988) for year-class 1981 than for the three other year-classes examined.

Surplus production, expressed per capita (i.e., per fish), declined markedly in the late 1980s and part of this decline resulted from changes in the somatic growth component (Fig. 7). Production of the stock declined through the 1980s (Fig. 7A). While each fish (age-3 and older) produced on average more than 0.2 kg of surplus production up to 1985, surplus production was nil from 1986 onward (Fig. 7B). Fishing resulted in negative net production values of –0.2 kg after 1982. Catches averaged 0.18 kg/fish in 1974–1991 and

© 1999 NRC Canada

Fig. 4. Length of 6-year-old Atlantic cod in the northern Gulf of St. Lawrence and index of CIL core temperature over the first 5 years of life. Symbols represent the year when cohorts born in the period 1968–1991 reached age 6. The regression (r^2 = 0.25) was not significant (P = 0.31) when the number of observations (24) was corrected for autocorrelation (effective n = 6, df = 4). Reference lines shown were selected arbitrarily.

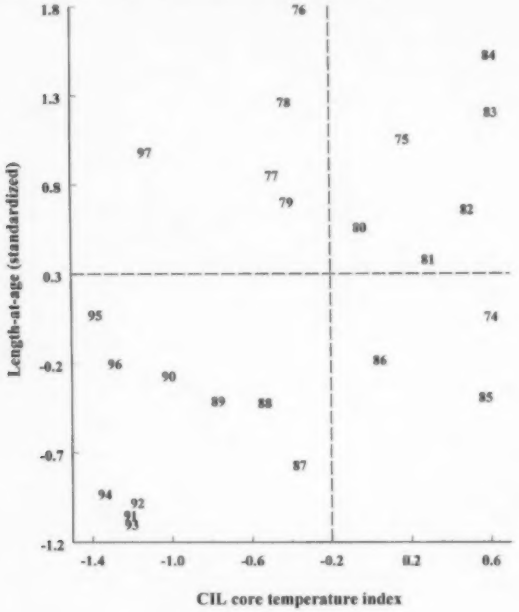
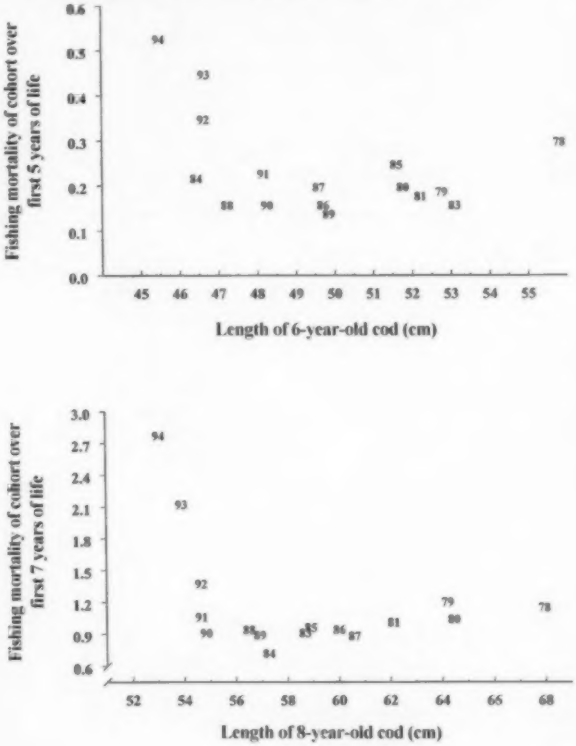


Fig. 5. Cumulative fishing mortality over the first 5 or 7 years of life for cohorts born in the period from 1970 to 1986 and resulting length of 8-year-old Atlantic cod in the period from 1978 to 1994 in the northern Gulf of St. Lawrence. Lengths are from the research surveys carried out in January, and age-specific fishing mortalities are from a recent assessment document (Fréchet and Schwab 1995). Fishing mortalities are not available prior to 1974 and commercial fishing was stopped in 1994. Symbols are years when lengths were measured.



0.30 kg/fish in 1992 and 1993 when fishing mortality increased dramatically (Fig. 5). Catch per capita did not differ between the periods 1974–1982 and 1983–1991 (P = 0.72). In contrast, the contribution of somatic growth to stock production was 0.25 kg/fish in 1974–1982 and only 0.15 kg/fish in 1983–1991 (P < 0.01). Losses to mortality (modeled as a doubling of M in Fréchet and Schwab 1998) also increased after 1985. Recruitment added 0.14 kg/fish to stock production annually in 1974–1991 and did not vary significantly between 1974–1982 and 1983–1991.

Length-at-age varies considerably among stocks in the North Atlantic, with cod in the Gulf of St. Lawrence being smaller than cod in most other stocks (Table 3). The mean length of 4-year-old cod varied from 41 to 67 cm, while that of 8-year-old cod varied from 52 to 96 cm in 1991. Georges Bank cod grew 65% faster than northern and southern Gulf of St. Lawrence cod between ages 4 and 8. Not all stocks are listed in Table 3, but no other stock was found with lengths-at-age shorter than cod in the Gulf of St. Lawrence.

Relationships between length-at-age and bottom temperature are poor for stocks living in cold water. The range of sizes at age 6 in 1991 was nearly 12 cm for a range of bottom temperatures of only 0.5°C for stocks living at temperatures below 3°C (Fig. 8A). Eastern Scotian Shelf cod are much smaller than northeast Arctic cod, which live at similar estimated ambient temperatures (4.5 and 4.0°C, respectively). The relationship between length at age 6 and temperature was significant when calculated for the 17 stocks combined (r^2 = 0.67, P < 0.01, n = 17) (see Brander 1995) and was also significant when calculated for stocks living at 5°C and above (r^2 = 0.62, P = 0.01, n = 9). However, this relationship was not significant when stocks living below 5°C were examined (r^2 = 0.07, P = 0.52, n = 8).

Deviations from this relationship between length-at-age and temperature resulted from differences in the ratio of actual to maximum growth rate. The instantaneous growth rate of 1-kg cod, based on weights-at-age in landings and calculated over 1 year, i.e., their size-specific age-independent growth rate (G), followed a similar pattern of variation as length-at-age for 6-year-old cod but represented a variable proportion of the maximum achievable instantaneous growth rate (G_{max}) (Fig. 8B). The growth rate of cod showing a positive deviation in the size or growth rate – temperature relationship represented a larger proportion of the maximum achievable rate (LGBK, SGBK, SNFD, and NEAC). In contrast, stocks showing a negative deviation from this general relationship had growth rates that represented a smaller proportion of the maximum growth rate expected for a given

© 1999 NRC Canada

Table 2. Comparison of mean back-calculated lengths-at-age of 8-year-old Atlantic cod caught during the January research survey with weighted mean measured lengths-at-age of cod caught in the same survey for corresponding year-classes (e.g., 1973 year-class: length of age-8 cod caught in 1981 was back-calculated to age 5 (48.0 cm) and compared with length of age-5 cod caught in January 1978 (48.4 cm)).

			Length (cm)				
Year-class	Year caught	Method	Age 4	Age 5	Age 6	Age 7	Age 8[a]
1973	1981	Back-calculated	39.1	48.0	54.5	60.4	64.7
		Measured	No data	48.4	52.8	58.3	62.1
1976	1984	Back-calculated	34.4	43.5	51.5	57.7	61.7
		Measured	35.5	44.0	No data	57.2	57.3
1981	1989	Back-calculated	33.5	42.2	48.4	53.5	56.8
		Measured	38.0	44.5	49.6	52.1	56.9
1986	1994	Back-calculated	33.2	41.7	48.2	53.3	57.2
		Measured	35.2	42.2	46.6	51.2	53.1

[a]Length shown for the back-calculated sample at age 8 is the measured length.

Fig. 6. Geometric mean regression of Atlantic cod body length (centimetres) against otolith radius (micrometres) (n = 279). Length and radius values are divided by their respective standard deviations.

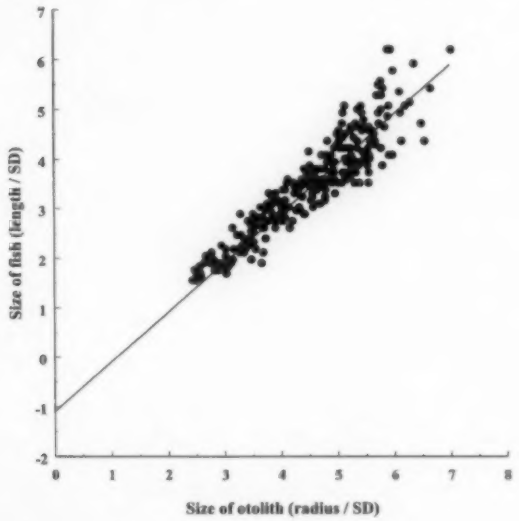
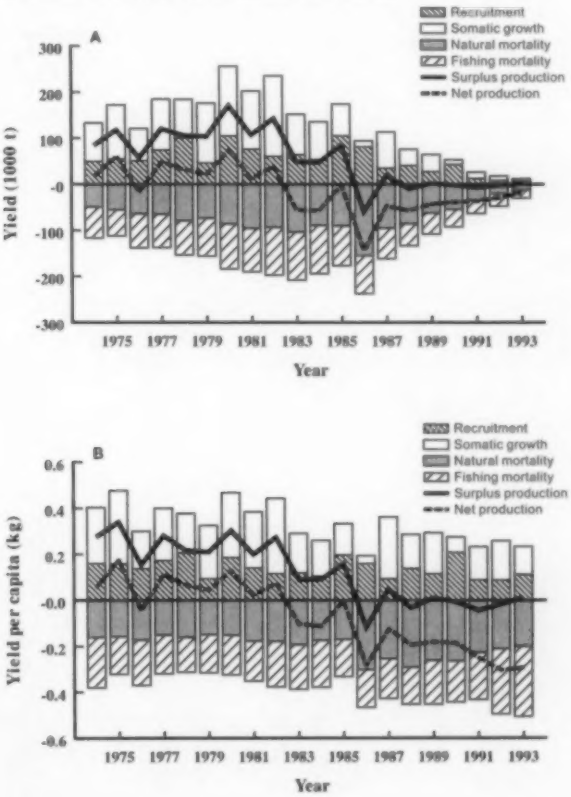


Fig. 7. Biological production of 3-year-old and older Atlantic cod in the northern Gulf of St. Lawrence. The increase in biomass due to growth and recruitment and the loss of biomass due to natural mortality and fishing mortality are reported as (A) total yield and (B) yield per capita, i.e., divided by stock size. The reported catch biomass is added to the net production to obtain the surplus production for the stock.



temperature (SGSL and ESSF). This observation also applies when we restrict our analysis to stocks living at similar temperatures (LGBK, SGBK, and SNFD versus NGSL and SGSL).

Stocks with fast growth rates and large sizes by age 6 tend to have higher condition factor values than stocks living in colder water (Fig. 9). Condition factor and growth rate data were examined for several stocks for which size at age 6 ranged from less than 1 to as much as 12 kg. Condition factor values formed two distinct clusters. Condition factors above 1.05 were common in fast-growing cod and exceptional in slow-growing cod. The variability in condition factor values also increased in slow-growing cod. Condition factor ranged from 0.84 to 1.03 in Gulf of St. Lawrence (NGSL and SGSL) cod in research surveys conducted in August and September 1972–1996.

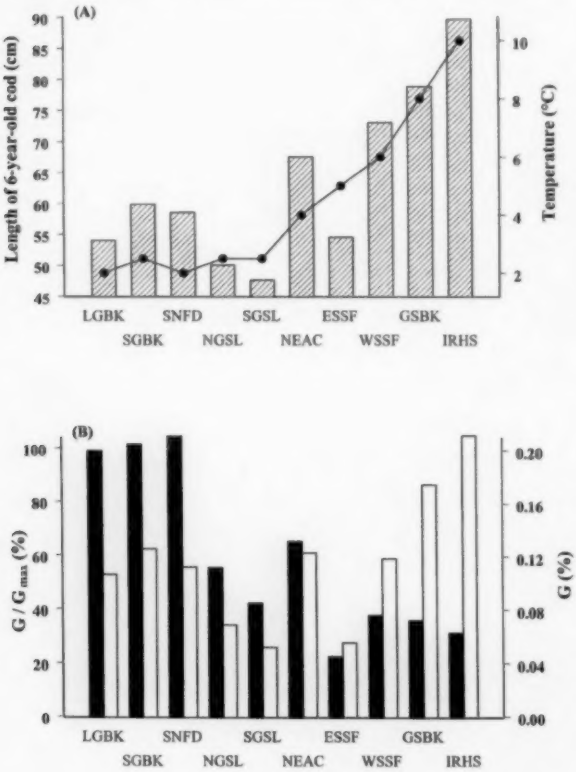
© 1999 NRC Canada

Table 3. Mean length (cm) of 4- to 8-year-old Atlantic cod in commercial catches landed in 1991 in the North Atlantic.

Stock	Fishing area	Age 4	5	6	7	8	Reference
Northern Gulf of St. Lawrence	3Pn, 4R, and 4S	41.3	45.6	50.0	53.9	56.6	Fréchet and Schwab 1995
Southern Gulf of St. Lawrence	4T and 4Vn	41.3	45.0	47.6	50.5	52.7	Currie and Sinclair 1996
Labrador and Grand Bank	2J, 3K, and 3L	41.0	47.7	54.0	59.2	62.5	Baird et al. 1992*a*
Eastern Scotian Shelf	4Vs and 4W	46.1	50.4	54.5	55.7	59.9	Mohn and MacEachern 1992
Southern Newfoundland	3Ps	42.8	50.0	58.5	64.9	68.8	Bishop and Murphy 1992
Southern Grand Bank	3NO	49.7	53.4	59.8	70.8	77.7	Baird et al. 1992*b*
Northeast Arctic	I	53.5	60.1	67.5	75.5	81.4	Ozhigin et al. 1995
Western Scotian Shelf	4X	59.1	65.2	73.0	81.8	91.0	Campana and Hamel 1992
Georges Bank	5Zjm	67.2	73.3	78.8	86.2	96.1	Hunt and Buzeta 1992

Note: Values were obtained from stock assessment documents. Fishing areas are from the Northwest Atlantic Fisheries Organization (NAFO) except for area I, which is from the International Council for the Exploration of the Sea (ICES).

Fig. 8. (A) Length of 6-year-old Atlantic cod (bars) in landings in 1991 and mean bottom temperature (line) to which various stocks are exposed in the North Atlantic. Lengths-at-age are from stock assessment documents and bottom temperatures are those of Brander (1995). (B) Instantaneous growth rate (G, open bars) and percentage of the maximum growth rate expected at a given temperature for a 1-kg cod (G_{max}, black bars) in various stocks in the North Atlantic: Labrador and Grand Bank (LGBK), southern Grand Bank (SGBK), Saint-Pierre Bank (SNFD), northern and southern Gulf of St. Lawrence (NGSL, SGSL), northeast Arctic (NEAC), eastern and western Scotian Shelf (ESSF, WSSF), Georges Bank (GSBK), and Irish Sea (IRHS).



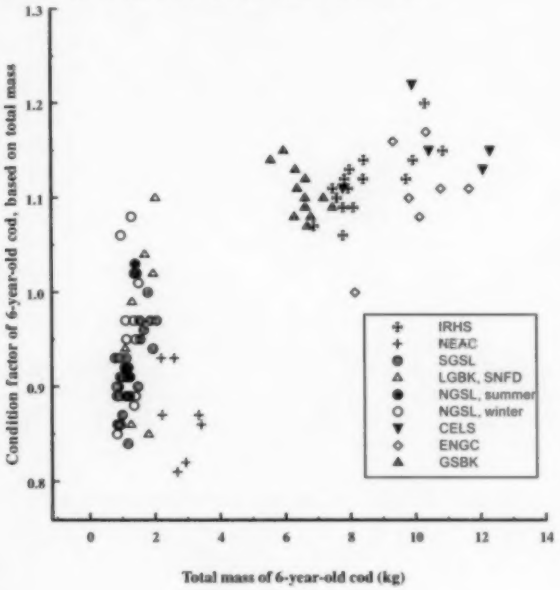
Discussion

There are many potential mechanisms resulting in variations in stock abundance, and complex interactions among numerous biotic and abiotic factors at the scale of large ecosystems make it unlikely that several stocks would vanish simultaneously unless equally large-scale perturbations occurred. Fishing mortality throughout eastern Canada was high enough to cause such perturbations, and it has been argued to be the single cause for the collapse of cod stocks (Hutchings and Myers 1994; Hutchings 1996). When fishing was closed in 1992–1994, stocks were expected to respond quickly to presumably favorable growth conditions resulting from low intraspecific competition. Recent assessments for northern cod indicate that such has not been the case. Similarly in the northern Gulf of St. Lawrence stock, abundance (3 years and older) was estimated to be 58 million fish in 1993 and 59 million fish in 1997 (Fréchet and Schwab 1998). Furthermore, several observations in the 1980s and 1990s, particularly with respect to growth (Chouinard and Fréchet 1994) and condition (Lambert and Dutil 1997*b*), were not consistent with the favorable growth conditions hypothesis. This suggested that declining sizes-at-age in northern Gulf cod in the 1980s resulted from real changes in growth rates, possibly stemming from changes in environmental conditions. We argue that slower growth rates in the second half of the 1980s and early 1990s led to a decline in stock productivity and that low productivity in turn made the stock more vulnerable to large and sustained fishing mortality.

Surplus production and net production per capita were markedly higher in the 1970s and early 1980s and became nil or negative in 1985 so that any amount of fishing would have caused a decline of the stock after 1985 with the rate of decline proportional to the magnitude of fishing mortality. For a similar level of exploitation, stock biomass was increasing when surplus production was high and decreasing when surplus production became nil or negative. Total allowable catches were lowered in the late 1980s in an effort to stop the decline and favor stock rebuilding, but net production remained negative. These results depend on SPA calibration. Fréchet and Schwab (1998) assumed that natural mortality has doubled (to 0.4) since 1986. Changes in natural mortality in SPA affect the age structure of the reconstructed population more than the estimates of abundance for the dominant age groups (age groups 6–8 in Fréchet and

© 1999 NRC Canada

Fig. 9. Condition factor and corresponding mass of 6-year-old Atlantic cod. NGSL, January and August research surveys; SGSL, September research surveys; NEAC, U.S.S.R. November–December research surveys; GSBK, LGBK, SNFD, CELS, IRHS, ENGC, commercial landings. Celtic Sea (CELS), English Channel (ENGC), see Fig. 8 for other stock codes.



Schwab 1998). An SPA based on lower natural mortality values will estimate a lower recruitment, however, since less fish are initially required to produce the measured abundance of survivors. Had we done an analysis with a different value of natural mortality, the distribution of production among components would have changed, but the overall trends would have remained similar for all but the most recent years, given the convergence properties of SPA (Pope 1972).

Discards are not factored in our analyses. Discarding of small fish and underreporting reached high levels in the 1980s, but no estimates exist about the magnitude of the problem, nor are there reasons to believe that discarding would have increased while cod abundance was declining. Discrepancies between SPA and survey trends in recruitment have been hypothesized to have resulted from increased discarding rates of small fish as fishing mortality increased (Myers et al. 1997). However, this is an unlikely scenario for cod in the northern Gulf of St. Lawrence. The opposite may actually have occurred: high incidence in the early to mid-1980s with high abundance, minimal enforcement, and limiting total allowable catches and then a gradual decline with declining fish abundance (total allowable catches stopped being limiting around 1987–1988), stricter enforcement, and such measures as increased mesh size and dockside monitoring. Rivard (1989) examined the various sources of bias in SPA. When underreporting is constant, population size and hence recruitment estimates are biased downward in a linear fashion with underreporting, but fishing mortality estimates are not affected. Underreporting is rarely constant with age and (or) time. When small fish are discarded, both population size and fishing mortality estimates for younger age groups will be biased downward. Since estimates of younger ages are not dependent only on estimates of catches for those age groups in SPA (basically, cumulative catches for all age groups in a cohort are used), recruitment estimates are not very sensitive to biases in catches at younger ages. If discarding is constant with time, the time trend in recruitment in the converged part of the matrix will be correct, but scaled down. Temporal changes in underreporting will introduce biases in fishing mortality and population size in the year previous to the change, an increase in misreporting will result in underestimating fishing mortality, and a decrease in misreporting will result in overestimating fishing mortality. Population sizes, and to a lesser extent recruitment, were probably underestimated in the 1980s relative to the 1990s. The main impact would be that population and recuitment were at higher levels than estimated in the 1980s relative to the 1990s, but on a per capita basis, neither recruitment nor growth production would be affected significantly (since underreporting acts by scaling down estimates), nor would be the loss through fishing mortality, since fishing mortality is not significantly affected by underreporting.

The low productivity of northern Gulf cod in the second half of the 1980s and in the 1990s is partly explained by a decline in growth production. This is consistent with changes not only in mean sizes-at-age but also in condition (Lambert and Dutil 1997*b*) during that period. There were differences in lengths-at-age as estimated from landings and the January and August research surveys, but all three sources of data indicate a significant decline in mean lengths-at-age of older cod. Sizes-at-age had declined in several stocks prior to their collapse in the 1990s. Smaller sizes-at-age have been documented for the northern and southern Gulf of St. Lawrence (Chouinard and Fréchet 1994), Labrador and Grand Bank (Lilly et al. 1998), eastern Scotian Shelf (Mohn and MacEachern 1992), east and west Greenland (ICES 1992*a*), and Faroe Plateau (Jákupsstovu and Reinert 1994). Sizes-at-age were smallest in the mid- to late 1980s and in the 1990s in the Gulf of St. Lawrence (Chouinard and Fréchet 1994) as well as in Labrador and on the Grand Bank (Lilly et al. 1998). Hutchings (1996) suggested that sizes-at-age in Labrador and on the Grand Bank were not unusually small in the late 1980s but instead were anomalously large in the late 1970s. Hutchings and Myers (1994) noted that the lowest weights-at-age for northern cod occurred between 1972 and 1975. Historical data on sizes-at-age of cod in the northern Gulf of St. Lawrence are scarce (May 1968; Pinhorn 1969; Fréchet and Schwab 1995) but show that lengths-at-age were above average in the mid-1970s and below average in the late 1980s. Lower growth production in the late 1980s cannot be ascribed to changes in the age and size structure of the stock. On average, cod in landings were 4.39 years old in 1978 and 4.62 years old in 1990 with a similar age structure (Fréchet and Schwab 1998). Mean length was remarkably stable during that period (40.1 ± 1.7 cm, minimum 37.5 cm in 1984 and maximum 42.8 cm in 1983, no trend). Thus, dividing production by population biomass instead of population numbers yields very similar results.

Smaller lengths-at-age in the late 1980s and early 1990s could have resulted from the selective removal of fast-

© 1999 NRC Canada

growing cod by the fishery if fishing mortality had increased or if the selectivity of the fishing gears had changed over the years. Cumulative fishing mortality for a cohort did not increase and thus cannot explain the major changes in size-at-age observed during the 1980s. Similarly, changes in selectivity of the fishing gears are unlikely to account for smaller sizes-at-age. Minimal mesh sizes for trawlers varied from 105 to 114 mm depending on material until 1976 (French trawlers used 140-mm mesh), from 120 to 130 mm between 1977 and 1981, 130 mm (all materials) from 1981 to 1993, and 130 mm square (145-mm diamond) since 1993. Regulated mesh (or hook) size for other gears did not change during the 1978–1991 period. However, proportions of cod landed by the various gears did change. Landings by trawlers rose from 45 to 75% (age of full recruitment 7; Fréchet and Chouinard 1987), whereas landings by longlines and gill nets (age of full recruitment 8 or 9) and by small-meshed traps and shrimp trawls (age of full recruitment 5) decreased from 42 to 22% and from 13 to 3%, respectively. The various changes in the fishery seem neutral as far as selectivity is concerned, with decreased landings by longlines and gill nets being balanced by the larger mesh size of otter trawls and a major decrease in landings by small-meshed gears. An examination of the gear-specific partial recruitments by Fréchet and Chouinard (1987) shows that the average partial recruitment (weighted by catch) remained remarkably constant for ages 4–7 in the 1980s.

Several factors in the environment can potentially explain declining sizes-at-age, including lower temperatures. A recent study (Gilbert and Pettigrew 1997) and our own data show that Gulf waters exhibit long-term trends in climatic conditions. There has been a major cooling event of Gulf waters from the above-average conditions of the early 1980s to the below-average conditions of the early 1990s, i.e., as much as a 2°C decline over the bulk of the water column. This situation may have resulted in environmental conditions less favorable for growth that could explain smaller sizes-at-age and lower growth production (per capita): weight of 6-year-old cod decreased 27% on average (January research survey, 1980–1991), while the maximum instantaneous growth rate is expected to decrease 27% for a 2°C decrease in temperature (from 4°C in 1980 to 2°C in 1991) (Jobling 1988). Temperature explained a non-negligible proportion of the variance, considering the likelihood of having several factors involved, and slopes were always positive, suggesting generally faster growth at higher temperatures. When accounting for autocorrelation, however, the relationship between temperature and size-at-age was not significant over the whole period of observation (1974–1997), suggesting that other factors must also be affecting length-at-age.

Nonsignificant correlations between size-at-age and temperature indices may be explained by several factors. Cod may have avoided cooler temperatures by changing their distribution. Castonguay et al. (1999) did not detect changes in the ambient temperatures of cod for the months of January (≈5°C) and August (≈2°C) in the northern Gulf of St. Lawrence, with cod being distributed 200 m deeper in January during the 1990s (450 m) than earlier (250 m). On the other hand, cooler temperatures may have affected cod through changes in the distribution and production of prey items. This would explain the low G/G_{max} ratio for the stock. Finally, we used tests with low statistical power. Corrections for autocorrelation resulted in very low degrees of freedom (3–6), and testing the coherence between two 10-year period signals generally requires longer time series.

Nevertheless, other observations suggest that climatic or environmental conditions may have had a negative impact on northern Gulf cod. Smaller sizes-at-age coincided with poor condition (Lambert and Dutil 1997*b*), suggesting that cod and (or) their preys were experiencing unfavorable environmental conditions. Back-calculated growth trajectories of cod having survived to age 8 confirmed that sizes-at-age changed in the 1980s, with the 1981 and 1986 year-classes having experienced slower growth rates. There was no evidence that ambient temperatures of cod during winter and summer surveys changed with the cooling of the Gulf, but the cooling seems to have resulted in an earlier fall migration to wintering grounds. A southerly shift of the median latitude of the winter cod distribution was noted that was positively correlated with the CIL core temperature index when accounting for autocorrelation (Castonguay et al. 1999). This was interpreted to indicate that cod left autumn feeding grounds progressively earlier in the late 1980s – early 1990s in response to the CIL cooling, which may have shortened the fall feeding season. This is consistent with our finding that size-at-age and latitude of distribution in January covaried in the 1980s and 1990s. Large negative temperature anomalies occurred elsewhere in the Northwest Atlantic in the mid-1980s and early-1990s that were associated with poor growth rates (Colbourne et al. 1994; Mann and Drinkwater 1994; Krohn et al. 1997). Interestingly, cod stocks inhabiting the southern part of the species' range (western Scotian Shelf and Georges Bank) where the cooling event was not felt did not exhibit declines in size-at-age (Campana et al. 1995). Because sea bottom temperatures in the 1980s were comparable with temperatures reported historically, Hutchings and Myers (1994) and Hutchings (1996) rejected the possibility that the collapse of northern cod was due to an interaction between fishing and a deteriorating environment. However, they were referring to environmentally induced increases in natural mortality and based their conclusion on an examination of associations between stock size (from SPA) and temperature.

Density-dependent factors, in contrast, are not likely to have played a role in the recent period. If growth rates of cod were density dependent, low standing stock biomasses in the late 1980s and 1990s would have promoted faster growth rates and hence larger sizes-at-age in surviving cod. The reverse was observed in several stocks (Hanson and Chouinard 1992; Swain and Wade 1993; Krohn and Kerr 1997), and especially in the northern Gulf of St. Lawrence with a low growth production per capita in the late 1980s and 1990s and a positive slope describing the relationship between size index and stock biomass for the 1974–1997 period. Stock size decreased 10-fold (Fréchet and Schwab 1998), from 563 million fish in 1983 (when temperatures were highest) to 58 million fish in 1993 (when temperatures were lowest). This could suggest that factors promoting faster growth also promoted higher standing stock biomasses

© 1999 NRC Canada

in the late 1970s and early 1980s and that reduced sizes-at-age later on had little to do with higher densities. Low densities should also have stimulated an increase in individual condition. Declining sizes-at-age, on the contrary, coincided with poor individual condition in the early 1990s (Lambert and Dutil 1997*b*). Furthermore, smaller sizes-at-age associated with poor condition (Fig. 9 and Lambert and Dutil 1997*b*) resulted in an increase in natural mortality (Dutil and Lambert 1999). Jorgensen (1992) reported a similar sequence of events for northeast Arctic cod, which exhibited a decline in both length-at-age and weight-at-length starting in the mid-1980s.

Differences in size-at-age among cod stocks in the North Atlantic correlate well with bottom temperatures. Cod tolerate temperatures ranging from –1 to +18°C and exhibit maximum growth rates near 13.5°C (reviewed by Jobling 1988). Growth rates of fish are controlled primarily by ration and temperature. Thus, size, age of cod, and temperature tend to covary, with cod living in the warmer waters of Europe exhibiting faster growth rates than cod living in colder waters such as the Gulf of St. Lawrence (Brander 1995). Variations in size-at-age of cod in eastern Canada could be explained by temperature, which was a significant covariate of length at all ages (Campana et al. 1995). While such relationships among size, age of cod, and temperature hold true in general for the range of temperatures at which cod stocks are found, size-at-age varies considerably among stocks living in colder waters, with the length of 6-year-old cod varying between 47 and 60 cm among stocks living in a narrow range of ambient temperatures (2.0–2.5°C).

Differences in metabolic costs or food availability may explain part of the variability in size-at-age among stocks living in cold water. Some stocks grow at maximum rates expected at low temperatures. For instance, Labrador and Grand Bank cod grew close to the maximum rate achievable at 2°C in 1990–1991. Thus, an increase in food availability would not have had as much impact on growth rates in this stock as an increase in available or selected bottom temperatures. For instance, a 1°C increase in the temperature to which cod were exposed in 1990–1991 would have represented a 14% increase in mass (1677 g instead of 1475 g) and a 42.5% increase in annual weight increment (677 g instead of 475 g) for a 1-kg fish in Labrador and Grand Bank under nonlimiting food conditions. In contrast, several other stocks that also lived in cold water but realized a smaller fraction of the maximum achievable growth rate in 1990–1991 may have experienced lower food availability. Campana et al. (1995) considered eastern Scotian Shelf cod as food-limited. Capelin (*Mallotus villosus*) biomass and temperature explained 52% of the variability in cod condition in NAFO divisions 2J and 3K but only 23% of the variability in growth rate in 2J, 3K, and 3L (Krohn et al. 1997). Temperature was a better predictor of growth rates than capelin biomass, but models failed to show that cold waters limited growth rates in 2J and 3K, possibly because the relatively warm temperatures offshore were used in their analysis, while cod experienced colder temperatures during the nearshore summer growth period. However, growth rates were limited by temperature in the colder areas (Krohn et al. 1997). The lower food availability hypothesis is consistent with our finding that cod with slow growth rates tend to have lower and much more variable condition factors than stocks living in warm waters. On the other hand, part of this variability among stocks living in cold water might be ascribable to uncertainties in the determination of ambient temperatures. Data on the distribution of cod relative to temperature are lacking, and temperatures used by ourselves and others are a crude estimate of temperatures actually experienced by cod. Values of G_{max} calculated for stocks living in warm temperatures may also be unrealistic, as cod might not be able to reach those rates even at maximum consumption. This would not invalidate comparisons for cold-water stocks living at similar temperatures, however.

The current management strategy for cod stocks under Canadian jurisdiction ignores overall stock production. Northern Gulf cod as well as several stocks in eastern Canada cannot experience fast growth rates because they live at low temperatures. Furthermore, the Gulf of St. Lawrence has been experiencing cooler than average water temperatures in the late 1980s and early 1990s. During this period, growth, a key component of production, followed trends in CIL temperature and was responsible along with increased mortality for low surplus production. Some stocks are evidently less productive than others and for this reason will not be able to withstand comparably high fishing mortality. We suggest that a decrease in biological production was one of the factors involved in the collapse of the northern Gulf cod stock. Our results argue in favor of new management practices that consider variations in productivity within and among stocks. This could be achieved by adjusting fishing mortality on size-at-age, less intensive rates of exploitation being exerted on more fragile, less productive stocks, such as the northern Gulf of St. Lawrence stock. This new management scheme should also include conservation measures whenever sizes-at-age decrease (an early warning signal) and condition deteriorates (a late warning signal that may be triggered seasonally or annually) below a threshold level. These factors must be taken into consideration when managing fisheries in variable environments.

Acknowledgements

Many persons have contributed to different aspects of this publication and we thank them sincerely. A. Fréchet provided data from the research surveys, the commercial fishery in the northern Gulf of St. Lawrence, and the recent SPA. G. Lilly retrieved and sent us a collection of cod otoliths for the years 1978–1981. S. Campana, G. Chouinard, L. Currie, P. Fanning, and A. Sinclair provided length and weight data for other Canadian stocks. M.J. Boon provided lengths and weights at age 6 for the Irish Sea, Celtic Sea, Bristol Channel, and English Channel cod. Y. Gagnon and J.-L. Beaulieu performed growth back-calculations. Most of the calculations involving the Atlantic Fisheries Adjustment Program database of temperature and salinity were performed by Y. Gauthier during summer 1995 as part of the Programme fédéral d'emplois d'été pour étudiants. W.E. Ricker and A. Sinclair advised us on back-calculations. L. Devine proofed the manuscript. This study was conducted under two projects funded by the Department of Fisheries and Oceans and

© 1999 NRC Canada

initiated following the collapse of several cod stocks in eastern Canada: a high priority project called "Partitioning the total mortality of Atlantic cod" and a special project at Institut Maurice-Lamontagne called "Programme de recherche multidisciplinaire sur la morue."

References

Baird, J.W., Bishop, C.A., Brodie, W.B., and Murphy, E.F. 1992*a*. An assessment of the cod stock in NAFO divisions 2J3KL. DFO Can. Atl. Fish. Sci. Advisory Comm. Res. Doc. 92/75.

Baird, J.W., Bishop, C.A., and Murphy, E.F. 1992*b*. An assessment of the cod stock in NAFO Div. 3NO. NAFO SCR Doc. 92/75.

Bishop, C.A., and Murphy, E.F. 1992. An assessment of the cod stock in NAFO subdivision 3Ps. DFO Can. Atl. Fish. Sci. Advisory Comm. Res. Doc. 92/111.

Brander, K.M. 1995. The effect of temperature on growth of Atlantic cod (*Gadus morhua*). ICES J. Mar. Sci. **52**: 1–10.

Campana, S., and Hamel, J. 1992. Status of the 1991 4X cod fishery. DFO Can. Atl. Fish. Sci. Advisory Comm. Res. Doc. 92/46.

Campana, S.E., Mohn, R.K., Smith, S.J., and Chouinard, G.A. 1995. Spatial implications of a temperature-based growth model for Atlantic cod (*Gadus morhua*) off the eastern coast of Canada. Can. J. Fish. Aquat. Sci. **52**: 2445–2456.

Castonguay, M., Rollet, C., Fréchet, A., Gagnon, P., Gilbert, D., and Brêthes, J.-C. 1999. Distribution changes of Atlantic cod (*Gadus morhua*) in the northern Gulf of St. Lawrence in relation to an oceanic cooling. ICES J. Mar. Sci. **56**: 333–344.

Chouinard, G.A., and Fréchet, A. 1994. Fluctuations in the cod stocks of the Gulf of St. Lawrence. ICES Mar. Sci. Symp. **198**: 121–139.

Colbourne, E., Narayanan, S., and Prisenberg, S. 1994. Climatic changes and environmental conditions in the Northwest Atlantic, 1970–1993. ICES Mar. Sci. Symp. **198**: 311–322.

Colbourne, E., de Young, B., Narayanan, S., and Helbig, J. 1997. Comparison of hydrography and circulation on the Newfoundland Shelf during 1990–1993 with the long-term mean. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 68–80.

Currie, L.G., and Sinclair, A.F. 1996. Catch-at-age for southern Gulf of St. Lawrence cod: 1971 to 1995. Can. MS Rep. Fish. Aquat. Sci. No. 2389.

Dutil, J.-D., and Lambert, Y. 2000. Natural mortality from poor condition in Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **57**. In press.

Dutil, J.-D., Lambert, Y., Chouinard, G.A., and Fréchet, A. 1995. Fish condition: what should we measure in cod (*Gadus morhua*). DFO Atl. Fish. Res. Doc. 95/11.

Fleming, A.M. 1960. Age, growth and sexual maturity of cod (*Gadus morhua*) in the Newfoundland area, 1947–1950. J. Fish. Res. Board Can. **17**: 775–809.

Fréchet, A., and Chouinard, G.A. 1987. Recrutements partiels, biomasses exploitables et rendements observés des diverses flottes de pêche à la morue du Golfe du Saint-Laurent (3Pn, 4RS, 4TVn, jan. à avr.). DFO Can. Atl. Fish. Sci. Advisory Comm. Res. Doc. 87/98.

Fréchet, A., and Schwab, P. 1992. Assessment of the northern Gulf of St. Lawrence cod stock (3Pn, 4RS) in 1991. DFO Can. Atl. Fish. Sci. Advisory Comm. Res. Doc. 92/77.

Fréchet, A., and Schwab, P. 1995. Review of the status of the northern Gulf of St. Lawrence cod stock (3Pn, 4RS) in 1994. DFO Atl. Fish. Res. Doc. 95/53.

Fréchet, A., and Schwab, P. 1998. Evaluation de la morue du nord du Golfe du Saint-Laurent (3Pn, 4RS) en 1997/Assessment of the Northern Gulf of St. Lawrence cod stock (3Pn, 4RS) in 1997. DFO Can. Stock Assess. Sec. Res. Doc. 98/127.

Fréchet, A., Gagnon, Y., Schwab, P., D'Amours, D., Dutil, J.-D., Lambert, Y., Bourassa, L., and Rollet, C. 1994. Review of the status of the northern Gulf of St. Lawrence cod stock (3Pn, 4RS) in 1993. DFO Atl. Fish. Res. Doc. 94/29.

Gavaris, S., and Gavaris, C.A. 1983. Estimation of catch at age and its variance for groundfish stocks in the Newfoundland region. *In* Sampling commercial catches of marine fish and invertebrates. *Edited by* W.G. Doubleday and D. Rivard. Can. Spec. Publ. Fish. Aquat. Sci. No. 66. pp. 178–182.

Gilbert, D., and Pettigrew, B. 1997. Interannual variability (1948–1994) of the CIL core temperature in the Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 57–67.

Gilbert, D., Vézina, A.F., Pettigrew, B., Swain, D.P., Galbraith, P.S., Devine, L., and Roy, N. 1997. État du golfe du Saint-Laurent: conditions océanographiques en 1995. Rapp. Tech. Can. Hydrog. Sci. Océaniques No. 191.

Hanson, J.M., and Chouinard, G.A. 1992. Evidence that size-selective mortality affects growth of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. J. Fish Biol. **41**: 31–41.

Hawkins, A.D., Soofiani, N.M., and Smith, G.W. 1985. Growth and feeding of juvenile cod (*Gadus morhua*). J. Cons. Int. Explor. Mer, **42**: 11–32.

Hunt, J.J., and Buzeta, M.-I. 1992. Status of the Atlantic cod stock on Georges Bank in unit areas 5Zj and 5Zm, 1978–1991. DFO Can. Atl. Fish. Sci. Advisory Comm. Res. Doc. 92/48: 23 pp.

Hutchings, J.A. 1996. Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. Can. J. Fish. Aquat. Sci. **53**: 943–962.

Hutchings, J.A., and Myers, R.A. 1994. What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. **51**: 2126–2146.

ICES. 1992*a*. The North-Western Working Group. ICES Working Group Rep. C.M. 1992/Assess: 14.

ICES. 1992*b*. Report of the Arctic Fisheries Working Group. ICES Working Group Rep. C.M. 1992/Assess: 2.

ICES. 1992*c*. Report of the Irish Sea and Bristol Channel Working Group. ICES Working Group Rep. C.M. 1992/Assess: 1.

ICES. 1992*d*. Report of the Roundfish Working Group. ICES Working Group Rep. C.M. 1992/Assess: 4.

Jákupsstovu, S.H.í., and Reinert, J. 1994. Fluctuations in the Faroe Plateau cod stock. ICES Mar. Sci. Symp. **198**: 194–211.

Jenkins, G.M., and Watts, D.G. 1968. Spectral analysis and its applications. Holden-Day, Oakland, Calif.

Jobling, M. 1982. Food and growth relationships of the cod, *Gadus morhua*, with special reference to Balsfjorden, north Norway. J. Fish Biol. **21**: 357–371.

Jobling, M. 1988. A review of the physiological and nutritional energetics of cod, *Gadus morhua*, with particular reference to growth under farmed conditions. Aquaculture, **70**: 1–19.

Jorgensen, T. 1992. Long-term changes in growth of North-east Arctic cod (*Gadus morhua*) and some environmental influences. ICES J. Mar. Sci. **49**: 263–277.

Krohn, M.M., and Kerr, S.R. 1997. Declining weight-at-age in northern cod and the potential importance of the early years and size-selective fishing mortality. NAFO Sci. Counc. Stud. **29**: 43–50.

Krohn, M., Reidy, S., and Kerr, S. 1997. Bioenergetic analysis of the effects of temperature and prey availability on growth and condition of northern cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 113–121.

© 1999 NRC Canada

Lambert, Y., and Dutil, J.-D. 1997*a*. Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 104–112.

Lambert, Y., and Dutil, J.-D. 1997*b*. Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Can. J. Fish. Aquat. Sci. **54**: 2388–2400.

Lear, W.H., and Parsons, L.S. 1993. History and management of the fishery for northern cod in NAFO Divisions 2J, 3K, and 3L. *In* Perspectives on Canadian marine fisheries management. *Edited by* L.S. Parsons and W.H. Lear. Can. Bull. Fish. Aquat. Sci. No. 226. pp. 55–89.

Lilly, G.R. 1997. Size and condition of cod in divisions 2J+3KL during 1978–1996. NAFO SCR Doc. 97/62.

Lilly, G.R., Shelton, P.A., Brattey, J., Cadigan, N., Murphy, E.F., Stansbury, D.E., Davis, M.B., and Morgan, M.J. 1998. An assessment of the cod stock in NAFO Divisions 2J+3KL. DFO Can. Stock Assess. Sec. Res. Doc. 98/15.

Mann, K.H., and Drinkwater, K.F. 1994. Environmental influences on fish and shellfish production in the Northwest Atlantic. Environ. Rev. **2**: 16–32.

May, A.W. 1968. Biological data on cod from the summer fishery on the North Shore Strait of Belle Isle. Fish. Res. Board Can. Tech. Rep. No. 27.

Minet, J.-P. 1978. Dynamics and yield assessment of the northeastern Gulf of St. Lawrence cod stock. Int. Comm. Northwest Atl. Fish. Sel. Pap. **3**.

Mohn, R., and MacEachern, W.J. 1992. Assessment of 4VsW cod in 1991. DFO Can. Atl. Fish. Sci. Advisory Comm. Res. Doc. 92/54.

Morgan, M.J., and Hoenig, J.M. 1997. Estimating maturity-at-age from length stratified sampling. J. Northwest Atl. Fish. Sci. **21**: 51–63.

Myers, R.A., and Cadigan, N.G. 1995. Was an increase in natural mortality responsible for the collapse of northern cod? Can. J. Fish. Aquat. Sci. **52**: 1274–1285.

Myers, R.A., Hutchings, J.A., and Barrowman, N.J. 1997. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecol. Appl. **7**: 91–106.

Ozhigin, V.K., Tretyak, V.L., Yaragina, N.A., and Ivshin, V.A. 1995. Growth of Arcto–Norwegian cod in dependence of environmental conditions and feeding. Theme session on causes of observed variations in fish growth. ICES CM1995/P:10.

Petrie, B., Drinkwater, K., Sandström, A., Pettipas, R., Gregory, D., Gilbert, D., and Sekhon, P. 1996. Temperature, salinity and sigma-*t* atlas for the Gulf of St. Lawrence. Can. Tech. Rep. Hydrogr. Ocean Sci. No. 178.

Pinhorn, A.T. 1969. Fishery and biology of Atlantic cod (*Gadus morhua*) off the southwest coast of Newfoundland. J. Fish. Res. Board Can. **26**: 3133–3164.

Pope, J.G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Int. Comm. Northwest Atl. Fish. Res. Bull. **9**: 65–74.

Pyper, B.J., and Peterman, R.M. 1998. Comparison of methods to account for autocorrelation in correlation analyses of fish data. Can. J. Fish. Aquat. Sci. **55**: 2127–2140.

Ricker, W.E. 1973. Linear regressions in fishery research. J. Fish. Res. Board Can. **30**: 409–434.

Ricker, W.E. 1979. Growth rates and models. *In* Fish physiology. Vol. 8. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 677–743.

Ricker, W.E. 1984. Computation and uses of central trend lines. Can. J. Zool. **62**: 1897–1905.

Ricker, W.E. 1992. Back-calculations of fish lengths based on proportionality between scale and length increments. Can. J. Fish. Aquat. Sci. **49**: 1018–1026.

Rivard, D. (*Editor*). 1982. APL program for stock assessments (revised). Can. Tech. Rep. Fish. Aquat. Sci. No. 1091.

Rivard, D. 1989. Overview of the systematic, structural and sampling errors in cohort analysis. Am. Fish. Soc. Symp. **6**: 49–65.

Sheridan, A.K. 1995. The genetic impacts of human activities on wild fish populations. Rev. Fish. Sci. **3**: 91–108.

Sinclair, A., Chouinard, G., Swain, D., Nielsen, G., Hanson, M., Currie, L., Hurlbut, T., and Hébert, R. 1995. Assessment of the southern Gulf of St. Lawrence cod stock, March 1995. DFO Atl. Fish. Res. Doc. 95/39.

Sinclair, A., Chouinard, G., and Currie, L. 1996. Assessment of the southern Gulf of St. Lawrence cod stock, March 1996. DFO Atl. Fish. Res. Doc. 96/52.

Smedstad, O.M., and Holm, J.C. 1996. Validation of back-calculation formulae for cod otoliths. J. Fish Biol. **49**: 973–985.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry. W.H. Freeman and Company, New York.

Swain, D.P., and Wade, E.J. 1993. Density-dependent geographic distribution of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **50**: 725–733.

Taggart, C.T., Anderson, J., Bishop, C., Colbourne, E., Hutchings, J., Lilly, G., Morgan, J., Murphy, E., Myers, R., Rose, G., and Shelton, P. 1994. Overview of cod stocks, biology, and environment in the Northwest Atlantic region of Newfoundland, with emphasis on northern cod. ICES Mar. Sci. Symp. **198**: 140–157.

Thompson, H. 1943. A biological and economic study of cod (*Gadus callarias*) in the Newfoundland area, including Labrador. Res. Bull. (Fish.) No. 14. Newfoundland Government, Department of Natural Resources, St. John's.

Thompson, R.O.R.Y. 1979. Coherence significance levels. J. Atmos. Sci. **36**: 2020–2021.

Waiwood, K.G., Smith, S.J., and Petersen, M.R. 1991. Feeding of Atlantic cod (*Gadus morhua*) at low temperatures. Can. J. Fish. Aquat. Sci. **48**: 824–831.

Wiles, M., and May, A.W. 1968. Biology and fishery of the West Newfoundland cod stock. Int. Comm. Northwest Atl. Fish. Res. Bull. **5**.

© 1999 NRC Canada

Concentration of capelin (*Mallotus villosus*) in tidal upwelling fronts at the head of the Laurentian Channel in the St. Lawrence estuary

Caroline Marchand, Yvan Simard, and Yves Gratton

Abstract: In 1994 and 1995, continuous monitoring of physical characteristics and echo integration were used to detect aggregations of capelin (*Mallotus villosus*) in the upper water column in relation to thermal fronts and to examine the tidal dynamics in an intense upwelling area at the head of the Laurentian Channel. During the four cruises, fish aggregations were recorded for 72% of the fronts (n = 61) resulting from the periodic upwelling of cold waters. Over the tidal cycle, only a low fish biomass was detected when the cold intermediate waters (< 2°C) upwelled during high tide; 90% of the 2-year-old capelin biomass was detected in water temperatures >2°C. Capelin zooplankton prey were concentrated primarily over the deep Laurentian Channel, with very small numbers being found in the shallower areas nearby, where the fronts occurred. The prey concentrations did not increase at the fronts at any phase of the tidal cycle. The few zooplankton patches detected by the optical plankton counter did not correspond to either physical structures or fish concentrations. The gut fullness index and the stomach contents of the capelin caught in fronts did not differ from those of capelin caught elsewhere. These frontal aggregations of capelin seem therefore to be driven more by a threshold response to low temperatures than by trophic interactions based on a higher prey density.

Résumé : En 1994 et 1995, le monitorage continu des caractéristiques physiques et l'échointégration furent employés pour détecter des agrégations de capelan (*Mallotus villosus*) dans la partie supérieure de la colonne d'eau en relation avec des fronts thermiques, ainsi que pour examiner la dynamique sur un cycle de marée dans une région de résurgence à la tête du chenal Laurentien. Au cours des quatre missions, des agrégations de poissons ont été notées dans 72% des fronts croisés (n = 61), résultant de la résurgence périodique d'eau froide. Pendant le cycle de marée, peu de biomasse de poissons fut détectée à marée haute lors de la résurgence de la couche intermédiaire froide (< 2°C); 90% de la biomasse de capelan de 2 ans a été détectée dans les eaux plus chaudes que 2°C. Les proies zooplanctoniques du capelan étaient essentiellement concentrées dans le chenal Laurentien et généralement en nombre très faible au-dessus des zones moins profondes qui l'entourent et où les fronts se manifestent. Leur concentration n'a pas montré d'augmentation aux fronts à aucune phase de la marée. Les quelques concentrations de zooplancton détectées par le compteur optique de particules ne coïncidaient pas avec les structures physiques ni avec les concentrations de poissons. L'index de remplissage des estomacs et les contenus stomacaux des capelans pêchés dans les fronts ne différaient pas de ceux des capelans pêchés ailleurs. Ces agrégations frontales de capelan semblent être plus dictées par une réponse à un seuil de basses températures que par des interactions trophiques basées sur une densité de proies plus élevée.

Introduction

Patchiness of organisms in pelagic ecosystems is often linked to hydrographic mechanisms, such as convergence in frontal systems and wind-driven or tidally driven processes, which favour the presence of aggregations at predictable locations and (or) times (e.g., Olson and Backus 1985; Simard et al. 1986*a*; Nash et al. 1989). Aggregations of zooplankton or small fish often attract larger predators such as whales, which need to feed in areas where food is abundant and concentrated (Nemoto 1970). The head of the Laurentian Channel in the lower St. Lawrence estuary (LSLE) (Fig. 1) is a traditional whale feeding ground (Edds and MacFarlane 1987) located in an area characterized by intensive frontogenesis (Ingram 1976; 1985), clearly delineated by satellite thermal imagery (Gratton et al. 1988; Lavoie et al. 1996).

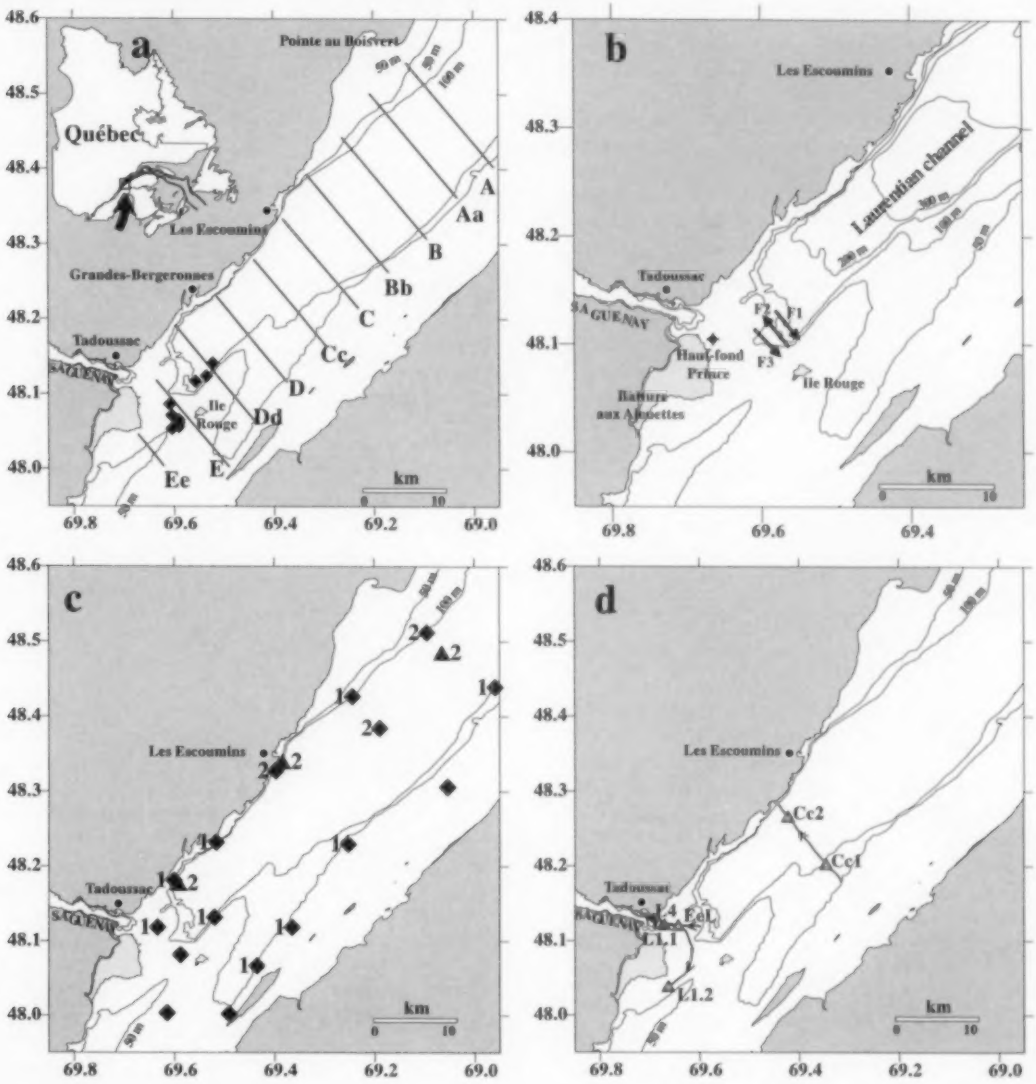
Received November 17, 1998. Accepted July 6, 1999.
J14898

C. Marchand. Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, QC G5H 3Z4, Canada, and Département d'Océanographie, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada.
Y. Simard.[1] Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, QC G5H 3Z4, Canada.
Y. Gratton.[2] INRS-Océanologie, 310 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada.

[1]Author to whom all correspondence should be addressed. e-mail: simardy@dfo-mpo.gc.ca
[2]Present address: INRS-Eau, 2800 Einstein, Sainte-Foy, QC G1C 4C7, Canada.

© 1999 NRC Canada

Fig. 1. Maps of the study area in the St. Lawrence estuary showing (*a*) the systematic parallel transects surveyed during the four cruises in 1994 and 1995 and the position of the trawl samples of cruise 4, (*b*) the three transects and their surveyed direction (arrows) on August 10, 1995, at the head of the Laurentian Channel, (*c*) the stations (▲, 1st survey; ◆, 2nd survey; 1, group 1; 2, group 2) where vertical zooplankton tows were done during cruise 4, and (*d*) the position of the fronts (▲) noted along the courses detailed in the results (survey direction indicated by arrows). Depth contours as indicated.



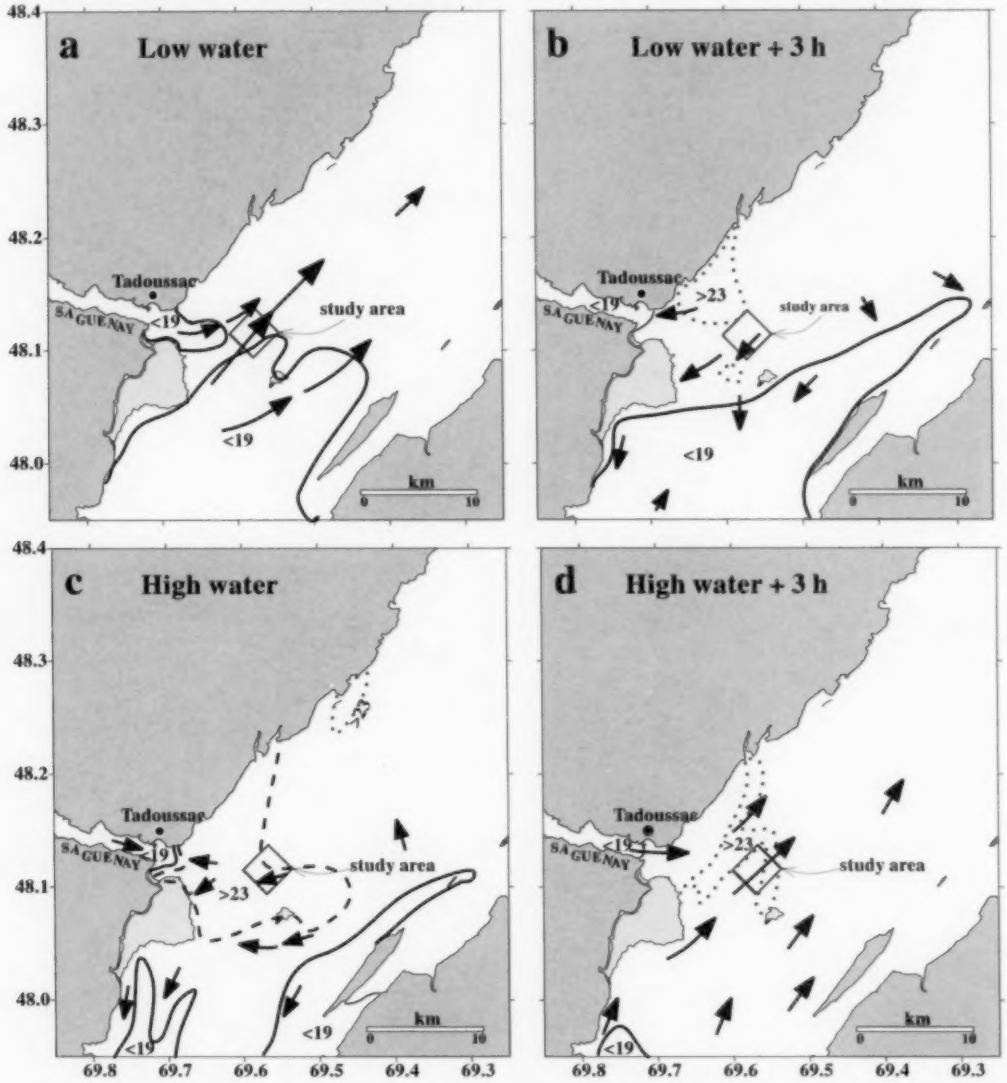
Do whale food resources aggregate at these fronts? If so, what are the mechanisms involved? These are the questions addressed in the present paper.

The bathymetry of the region is characterized by the shoaling (up to depth of ~20 m) of the 300-m-deep Laurentian Channel at a sill at the mouth of the Saguenay Fjord separating the lower estuary from the shallower (<150 m) upper estuary. Île Rouge, with its extensive shallow bank, is another local feature that forms a barrier that influences the region's hydrography. At the sill, the tides induce semi-diurnal upwelling of the lower estuary cold (temperature < 2°C) intermediate layer (CIL), which intrudes from the Gulf of St. Lawrence at depths of 70–90 m (Fig. 2) (Therriault and Lacroix 1976; Canadian Hydrographic Service 1997). At the channel head, three different water masses come into contact under a high-energy regime: the upper estuary surface waters, the Saguenay brackish waters, and the colder, more saline waters from the CIL.

Capelin (*Mallotus villosus*) (especially 1- and 2-year-old immatures) concentrate in the shallows at the head of the Laurentian Channel from April to November (Bailey et al. 1977). Aggregations were recorded in the vicinity of fronts

© 1999 NRC Canada

Fig. 2. Typical changes in the direction and strength of tidal currents (arrows) and density fields ($\sigma_t < 19$, $\sigma_t > 23$) at the head of the Laurentian Channel during a semidiurnal cycle (from Canadian Hydrographic Service 1997, fig. 6). The dashed line in Fig. 2*c* indicates more developed upwelling than the dotted lines in Figs. 2*b*–2*d*.



during flood tide at the mouth of the Saguenay (Laurin 1982). At the boundary between the different water masses, fish can be expected to react to the changes in their environment. Temperature is one of the important environmental variables that influence the temporal and spatial distribution of marine fish (Laevastu and Hayes 1981). Capelin are known to react to temperature at various scales. At large scales, the species can be found in water temperatures ranging from about −1.5 to 13.5°C in the Gulf of St. Lawrence (Bailey et al. 1977; Rose and Leggett 1989). At smaller spatial and temporal scales, the preferred temperature range is narrower. The lower temperature limit for most of the biomass appears to be well above 0°C during both spawning and feeding periods. Bailey et al. (1977), Rose and Leggett (1989), Methven and Piatt (1991), and Vilhjálmsson (1994) recorded the occurrence of most capelin schools above the 0°C isotherm, at depths less than 50 m, in various environments. The northern limit of the Barents Sea capelin distribution was found to correspond to the position of the 0°C isotherm at a depth of 100 m (Dommasnes and Røttingen 1985). Therefore, cold waters appear to limit both the vertical and horizontal distribution of capelin.

Many authors have invoked a threshold response to a low temperature limit to explain the coastal distribution and the catch rate of capelin in inshore traps in response to rising cold intermediate waters during strong upwelling events (Schneider and Methven 1988; Horne 1994; Schneider 1994; Ings et al. 1997). Capelin appear to tolerate a given range of

© 1999 NRC Canada

temperature, which varies in time and space; however, they seem sensitive to its lower limit, which appears to be above 0°C. Therefore, as postulated by Schneider and Methven (1988) and Horne (1994), horizontal concentrating of capelin can be expected in upwelling fronts, where a limiting isotherm would be brought to the surface and displaced horizontally. In this study, we used synoptic, high-resolution data in the horizontal and vertical planes, on both temperature and capelin distribution fields, to test this hypothesis in the tidally driven upwelling environment at the head of the Laurentian Channel. An alternative hypothesis, namely that the capelin aggregations are controlled by foraging behaviour related to the presence of high concentrations of food in the frontal systems, was investigated simultaneously. We also set out to determine the lower temperature limiting the local distribution of 2-year-old capelin. This work was done under a 4-year multidisciplinary research program on the dynamics of the whale feeding ground at the head of the Laurentian Channel in the St. Lawrence estuary.

Materials and methods

Sampling

Location of fronts in the study area and their effect on fish patchiness

A grid of 10 systematic, parallel cross-channel transects, 7.4 km apart (Fig. 1*a*), was visited eight times to locate the fronts in the study area and to check for biomass aggregations. In 1994, the monthly sampling periods were June 28 to July 4 (cruise 1), July 26 to August 1 (cruise 2), and August 24–30 (cruise 3). In 1995, sampling was done during August 7–18 (cruise 4). The grid was visited twice during each cruise, and each visit took 2 days. In the period between the two visits, additional ship tracks were sampled in sectors where strong fish echoes had been recorded, especially off Île Rouge. During cruises 1 and 3, transects Aa, Bb, Cc, and Dd were partially surveyed or omitted. Echo integration (Burczynski 1982; Johannesson and Mitson 1983) was used to map the fish and (or) macrozooplankton concentrations along the transects. A 120-kHz BioSonics model 102 echosounder equipped with a dual-beam (3.3 and 10°) transducer was connected to a computer via a BioSonics ESP (echo signal processing) interface to record the echo energy using ESP-EI version 3.0 software. Echo integration bins were partitioned into 5-m depth strata, starting 5 m below the surface; however, to achieve a higher resolution, bin thickness was reduced to 1 m beginning 30 m above the bottom. Horizontally, the integration was done over 20 pings (at a rate of $1 \cdot s^{-1}$), which corresponds to about 80 m at the 6- to 8-kn cruising speed along the transects. However, the integration unit was about 30 m for cruise 1 and the second visit of cruise 3 and about100 m for the off-transect tracks. Surface temperature, salinity (Applied Microsystems Ltd. STD-12, with response times of 85 and 25 ms), and light transmission (Sea Tech Inc. transmissometer, 25-cm path) were continuously measured in water pumped from a depth of about 2.5 m, and these measurements were recorded every 10 s along with the spatial position (Magnavox-200 GPS). Given the frontal width of a few kilometres and the short distance travelled by the ship in 10 s (<50 m), the horizontal resolution of the measures was high enough to prevent significant aliasing at the scale of the phenomena discussed in this paper. The actual gradients may, however, have been slightly smoothed and offset by the pumping system. Wind speed and wave amplitude (<2 m) were low during all surveys, which were conducted under flat, calm conditions for the most part. Clear internal wave trains, characterized by a series of parallel surface slicks a few hundred metres apart and echosounder traces showing vertical oscillations, were observed on a few occasions. This signature differed clearly from the frontal signature showing the boundary between two different water masses (e.g., see Fig. 6), although some fronts showed marked vertical movements on the echosounder.

Mesoscale tidal variability at an upwelling site

To determine the mesoscale variations in the physical and biological characteristics of the upper water column over a semidiurnal cycle, a special survey design was used off Île Rouge on August 10, 1995, 6 days after neap tide. Sampling was carried out along a grid of three parallel transects (~4 km long and 1 km apart) crossing the very end of the Laurentian Channel (Fig. 1*b*). The grid was visited five times over the semidiurnal tidal cycle: from low tide (07:56 EDT) to 3 h after high tide (17:48), referenced to Pointe-au-Père, 90 km downstream. A complete circuit consisted of (*i*) monitoring the surface characteristics and echosounding the water column along the three transects (F1, F2, and F3) at 6–8 kn, starting from the downstream transect (F1) and following the directions indicated by the arrows in Fig. 1*b*, which took about 1 h, and (*ii*) a second sampling of the central transect (F2b, "b" indicating the second sampling of transect F2 during one cycle) at 3–4 kn. During this sampling phase, a cross section of the physical characteristics and zooplankton biomass was obtained by towing (tow-yo) a vertically oscillating probe consisting of a Bongo net equipped with an OPC (Focal Technologies Inc.) and a CTD (Applied Microsystems Ltd. STD-12). Transect F2 was thus visited 10 times and transects F1 and F3 five times. The OPC, equipped with a 10× particle concentrator and an electronic flowmeter, was mounted in one opening of the Bongo net. The OPC and the onboard CTD were monitored in real time by a computer, which recorded the size of each particle and light attenuation (Herman 1992), together with GPS readings every second. Mesozooplankton samples were obtained from the net (333-μm mesh) placed in the second opening (60 cm in diameter) of the Bongo. The presence of front lines (presence of foam, change in water colour, flotsam accumulation), seabird concentrations, and whales was systematically recorded. Wind speed (northwest, 5–10 $m \cdot s^{-1}$) and wave amplitude (<1 m) were low throughout the day.

Fish and zooplankton sampling

During cruise 4, zooplankton samples were collected throughout the water column with the Bongo net from a grid of 19 stations (Fig. 1*c*). Fish in the upper 40 m were sampled on August 9 during the day and on August 10 during the night with a Marinovich pelagic trawl (codend mesh = 5 mm, opening = 3 × 5 m) capable of catching small nekton (krill and small pelagic fish) (Fig. 1*a*). Tows were made from a 20-m trawler and usually lasted 30 min. A maximum of about 200 fish were randomly selected from each catch. All zooplankton and fish samples were frozen (at –20°C) for later analysis.

Data analysis

In this study, we used Mann and Lazier's (1991) definition of a front: "...narrow regions where horizontal gradients are large....". We observed fronts with a wide variety of ranges, but the typical temperature (salinity) gradient was 2–5°C (2–6 PSU) over a few kilometres. Most of the fronts were tide induced (Ingram 1976, 1985); hence, their strength varies with the semidiurnal and fortnightly tidal cycles. From a preliminary examination of the data set, we found that thermal gradients larger than $0.5°C \cdot km^{-1}$ in the surface CTD data were a good indicator of the presence of fronts along the ship track. That is how we identified the fronts in the data set from the four cruises in the study area. We did not use surface ripples or accumulations of debris to determine the presence of fronts. To check for concentrations of fish and (or) macrozooplankton in the frontal zone, we examined the aggrega-

© 1999 NRC Canada

tion pattern on the echograms of mean volume backscattering strength (MVBS, square metres per cubic metre) in the upper 80 m (30 m on sections F1, F2, and F3) and looked at the change in MVBS computed over the first three vertical bins (depths of 5–20 m). The MVBS provides a measure of relative biomass density, using the parameters obtained from calibration with a standard sphere (Foote et al. 1987). The MVBS echograms were generated with SON software (Maurice Lamontagne Institute, Hydroacoustic Laboratory), a Windows® application designed to convert, edit, and display ESP-EI data from sampled ship tracks. To analyse the fine-scale distribution of biomass in a frontal area, high-resolution echograms were produced from the raw data stored on magnetic tapes using CH1 and CH2 data acquisition and visualization software (Simard et al. 1998). NOAA-12 and NOAA-14 satellite thermal images were obtained from the Remote Sensing Laboratory at the Maurice Lamontagne Institute (Lavoie et al. 1996).

OPC light transmission, CTD tow-yo, and surface CTD data were used to map the 0- to 30-m cross-channel physical characteristics on transect F2b over the tidal cycle. The kriging interpolation method, specifying the appropriate linear variogram for each data set, was used with a horizontally elongated neighbourhood. The OPC particles were grouped in 10-s intervals and sorted by equivalent spherical diameter classes (Herman 1992; Herman et al. 1993) using OPC-DAS software (Focal Technologies Inc. 1992). The diameter classes used were 1–4 mm for large copepods such as adult *Calanus finmarchicus* and 4–7 mm for larger zooplankton such as euphausiids and hyperids. The MVBS distribution relative to ambient temperature for F2b surveys was obtained by interpolating the temperature at the middle of each echo integration bin from the simultaneous tow-yo data.

Zooplankton samples were sorted for macrozooplankton (>5–6 mm) and then subsampled with a Folsom splitter (Van Guelpen et al. 1982) to determine species composition and total dry weight biomass (by drying at 60°C until constant weight). Cluster analyses (single linkage, complete linkage, and UPGMA; Legendre and Legendre 1998) were performed on nonstandardized zooplankton concentrations using the Bray–Curtis coefficient in order to group the zooplankton stations according to species assemblage.

The length frequency distribution of capelin was determined from thawed fish samples. A measure of stomach fullness was estimated using a six-point scale from 0 (stomach empty) to 5 (stomach full) to assess overall feeding intensity. Differences in capelin stomach fullness between catches in the vicinity (<1 km) of front lines and elsewhere (>1 km) were tested with a χ^2 analysis. A count of the total number of prey items in each prey category was also made for each stomach containing food. According to the method of Vésin et al. (1981), an index of importance by number of prey categories was then calculated using the formula $IN = \sqrt{(O \times C)}$, where O is percent prey occurrence in the stomachs and C is percent composition in number of given prey in one sample (i.e., the number of individuals of the prey type relative to the total number of prey items in the sample). Only samples with at least 10 fish were retained for this analysis.

Results

Fish and zooplankton samples

In keeping with the findings of Bailey et al. (1977), the catch made with the small-mesh pelagic trawl in the upper 40 m was almost exclusively composed of immature capelin (2 years old, mean total length = 129.3 ± 0.4 mm, n = 683), which represented 99.6% of captured fish. They were thus considered to be the main source of the echoes in the upper water column, although the trawl sampling done in the study area was very limited owing to technical difficulties (uneven bottom, strong current shear) and logistic constraints. The low concentration of macrozooplankton (e.g., krill) caught with the pelagic trawl or the Bongo net or counted by the OPC (Table 1; and see Fig. 7) could not account for the strong echoes observed. The centre of distribution of the krill scattering layer is further downstream and their daytime depth is greater than 40 m (Simard et al. 1986*a*, 1986*b*; Simard and Lavoie 1999).Therefore, we assume that the echoes recorded in the upper water column were essentially from capelin.

Most capelin stomachs (n = 646) contained a significant amount of food. More than 75.1% had a fullness index ≥3. The frequency distribution of the stomach fullness indices for the capelin caught in frontal areas did not differ significantly (χ^2 test, $p > 0.05$) from that for capelin caught elsewhere in the upper 40 m. The small sample size precludes further inference about possible feeding rhythms, but the daytime and nighttime samples did not show any difference in stomach fullness or content. The diet was always very similar and dominated by copepods (IN = 96.6), mostly *Calanus finmarchicus* (IN = 80.2) and *Metridia* spp. (IN = 50.9). Euphausiid larvae (IN_{larvae} = 17.3) and *Calanus hyperboreus* (IN = 13.1) occurred with moderate frequency. Adult euphausiids (IN_{adults} = 3.9), fish eggs (IN = 3.6), and hyperids (IN = 1.2) also made a slight contribution to the diet. Other prey categories were present in trace amounts (see Marchand 1997).

The 19 zooplankton stations were divided into two main groups (group 1: nine stations; group 2: six stations) and four singletons through the cluster analysis of their taxonomic composition (Fig. 1*c*; Table 1). All the clustering algorithms gave similar results. Group 1 stations were found to be located along the edges of the Laurentian Channel in water depths less than 60 m, except for the northern station (107 m) on transect B, northeast of Les Escoumins. These stations were characterized by low concentrations of all zooplankton taxa. The second cluster comprised the stations situated at depths of over 120 m in the Laurentian Channel. Their zooplankton concentrations per cubic metre were generally more than one order of magnitude larger than those of group 1 and differed significantly (one-tailed t test, $p < 0.05$) in terms of the older life stages of all copepod taxa, which were the main capelin prey items. The four singletons were situated in shallow waters outside the Laurentian Channel and had almost no zooplankton. Another cluster analysis grouped the zooplankton samples collected along transect F2b with group 1, where the total sampled depths were similar (Table 1). The bulk of zooplankton appeared to be confined to the Laurentian Channel, at depths greater than 60 m, with only low zooplankton concentrations present in the bordering shallows.

Location of fronts in the study area and their effect on fish patchiness

During the four cruises spanning 1994 and 1995, 61 fronts were sampled at all tidal phases, mostly upstream of Les Escoumins (Fig. 3) and especially between the mouth of the Saguenay and Île Rouge, in the area of the high-resolution study of August 10, 1995. The other areas of occurrence were (*i*) the north shore of the LSLE, (*ii*) the southern edge

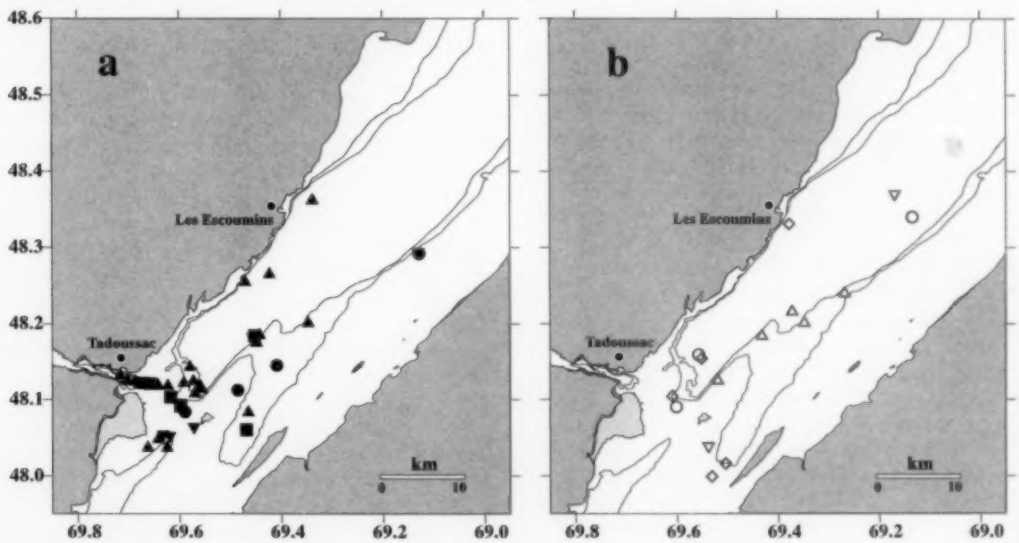
© 1999 NRC Canada

Table 1. Average density of zooplankton for the Bongo samples of the tow-yo surveys on August 10, 1995, and cruise 4 vertical hauls for the two groups of stations (Fig. 1*c*) presenting similar composition from the cluster analysis (no.·m^{-3} ± SD).

	Transect					Cluster analysis	
Species	$F2b_1$	$F2b_2$	$F2b_3$	$F2b_4$	$F2b_5$	Group 1	Group 2
Copepods							
Calanus finmarchicus (CI–CIII)	6.734	2.571	0.182	0.851	—	2.694±3.676	19.845±32.725
Calanus finmarchicus (CIV–CVI)	21.364	5.440	6.931	16.446	—	8.171±5.941	155.380±148.427
Calanus hyperboreus (CI–CIII)	0	0	0	0	—	0.010±0.024	1.474±2.201
Calanus hyperboreus (CIV–CVI)	0	0.060	0.061	0	—	0.702±1.226	17.857±12.023
Metridia sp. (CI–CIII)	0	0	0	0	—	0.060±0.114	2.763±2.746
Metridia sp. (CIV–CVI)	0	0	0.182	0	—	0.246±0.466	71.923±77.022
Euchaeta sp. (CI–CIII)	0	0	0	0	—	0	0
Euchaeta sp. (CIV–CVI)	0.232	0	0	0	—	0.016±0.045	1.199±1.137
Euphausiids							
Meganictyphanes norvegica	0	0.002	0	0	0	0	0.048±0.047
Thysanoessa raschi	0.001	0.002	0.001	0.001	0	0.121±0.352	0.761±1.496
Larvae	0.682	2.570	0	1.701	—	1.518±2.353	34.317±80.393
Amphipods							
Hyperids	0.036	0.019	0.045	0.120	0.045	0.121±0.248	0.097±0.106
Gammarids	0	0	0	0.045	0	0	0.000±0.001
Others							
Cnidarians	0	0	0	0.000	—	0.003±0.008	0.000±0.001
Ctenophores	0.001	0	0.001	0	—	0.003±0.007	0.001±0.002
Total dry weight (mg·m^{-3})	4.6	2.5	5.1	16.2	5.5	10.2±10.9	100.9±57.0
Filtered volume (m^3)	1102	1070	1052	902	842	107±61.3	310±180.3
Maximum sampled depth (m)	49	33	31	30	31	47±24	191±64

Note: —, small but not sorted because of mucilaginous remains; 0, absent.

Fig. 3. Distribution of fronts (*a*) with and (*b*) without aggregations of biomass that were detected at 120 kHz during the four cruises in 1994 and 1995. ◆ and ◇, cruise 1; ▼ and ▽, cruise 2; ● and ○, cruise 3; ▲ and △, cruise 4.



of the channel, and (*iii*) the southern arm of the channel (southeast of Île Rouge). The observations are biased in favour of the Île Rouge site because of the greater sampling effort. Sharp changes of up to 8°C in temperature and up to 9 units of salinity were noted, especially at the mouth of the Saguenay Fjord. The changes in light transmission were generally below 10% but reached 20% in some cases.

Aggregations of biomass occurred in more than 72% of the 61 front observations (Fig. 3*a*). The proportion of fronts with biomass aggregations was similar at all tidal phases:

© 1999 NRC Canada

Fig. 4. Examples of fronts observed in 1995 in the LSLE during cruise 4, located on Fig. 1*d*. Echogram of the MVBS, $MVBS_{5-20}$ and temperature, salinity, and transmittance at ~2.5 m. (*a*) Front L4 (~18:00, L + 5) on August 16, (*b*) fronts L1.1 (~07:20, H) and L1.2 (~8:00, H + 1) on August 17, (*c*) front EeL (~18:00, H + 5) on August 8, and (*d*) fronts Cc1 (~10:00, L + 3) and Cc2 (~10:30, L + 3) on August 8. Surface front lines are indicated (with seabirds (+B) when present). Transect direction is given on Fig. 1*d* and the *x*-axis gives the distance from the starting point. Note that vertical axes are not at the same scale for the physical characteristics and $MVBS_{5-20}$.

78% at high tide ±1 h (n = 27), 73% during flood (n = 11), 64% at low tide ±1 h (n = 11), and 75% during ebb (n = 12). Unfortunately, one-time sampling does not provide a basis for determining which tidal phase is more important for the formation of aggregations. In less than 5% of the front observations, fish concentrations were found in the colder water mass. Echo intensity was not related to the strength of the temperature or salinity gradients.

Six front crossings (Fig. 1*d*), where an increase in biomass was noted, are described below. Most of them had an accumulation of buoyant particles (fucus) at the surface indicating the presence of convergent currents. Front L4 (Fig. 4*a*) was observed at the sill in the Saguenay Fjord entrance at the end of flood tide on August 16, 1995. This front was characterized by surface temperature changes of 2–3°C and salinity changes of roughly 1.8 over a distance of about 2 km. As Fig. 2*c* indicates, the surface waters outside the Saguenay were denser at this tidal phase. During flood tide at this location, the dense LSLE waters plunge under the Saguenay waters and penetrate into the deep layer of the fjord. The light-scattering layer curving between 20 and 40 m over the sill (Figs. 4*a* and 5) is probably entrained by this downwelling. At the interface of the two water masses, the $MVBS_{5-20}$ peaked to 4.0×10^{-5} $m^2 \cdot m^{-3}$. This high biomass concentration was associated with a larger aggregation extending to a depth of 100 m in the basin at the fjord entrance (Fig. 5).

One day later 1 h after high tide, a front (L1.1, Figs. 1*d* and 4*b*) with comparable salinity and biomass distribution was observed at the same location. It corresponded to the Saguenay plume edge. On this ship track from Tadoussac to transect Ee, a second front (L1.2, Fig. 4*b*) was located off the Batture aux Alouettes. This was another zone of contact between upwelled LSLE waters and warmer (temperature ≥6.5°C), fresher (salinity ≤26.5) waters. The $MVBS_{5-20}$ increased up to 2.0×10^{-7} $m^2 \cdot m^{-3}$ at this frontal boundary but did not reach the concentration recorded on the previous day.

The same type of front was observed farther over the sill at the end of the ebb (front EeL, Figs. 1*d* and 4*c*). At this tidal phase (Fig. 2*a*), the Saguenay plume extended farther into the estuary. An $MVBS_{5-20}$ peak was also noted in this area in the more saline waters. This biomass aggregation extended into a scattering layer that plunged under the Saguenay plume as in the case of front L4. The front was also characterized by surface rippling. It was visible on a satellite image 7 h before the ship crossed it, but it then retreated a few kilometres towards the mouth of the Saguenay (Fig. 6). Several minke and fin whales were present in the frontal area.

Another type of front present at both ends of transect Cc on August 8, 1995, marked the boundary between a warm (temperature between 11 and 12°C), more limpid surface water mass and surrounding waters that were about 5°C colder. Higher biomass was observed at the interface between the two water masses in the upper 20 m of the water column. These aggregations were characterized by a maximum $MVBS_{5-20}$ of 2.0×10^{-6} $m^2 \cdot m^{-3}$ in front Cc1 and 1.2×10^{-5} $m^2 \cdot m^{-3}$ in front Cc2. Significant biomass was present in the warmer waters, but at a much lower concentration compared with the aggregations detected in both fronts. Both fronts were visible on a satellite image taken less than 1 h after the sampling done on the same day (Fig. 6).

There were only 17 occasions (28% of front observations) during the study when no particular biomass aggregations were observed in fronts (Fig. 3*b*). In some cases, little or no biomass was present in the surface waters of the frontal zone or along the transect. In other cases, biomass was present but without any distinct aggregation in the upper water column.

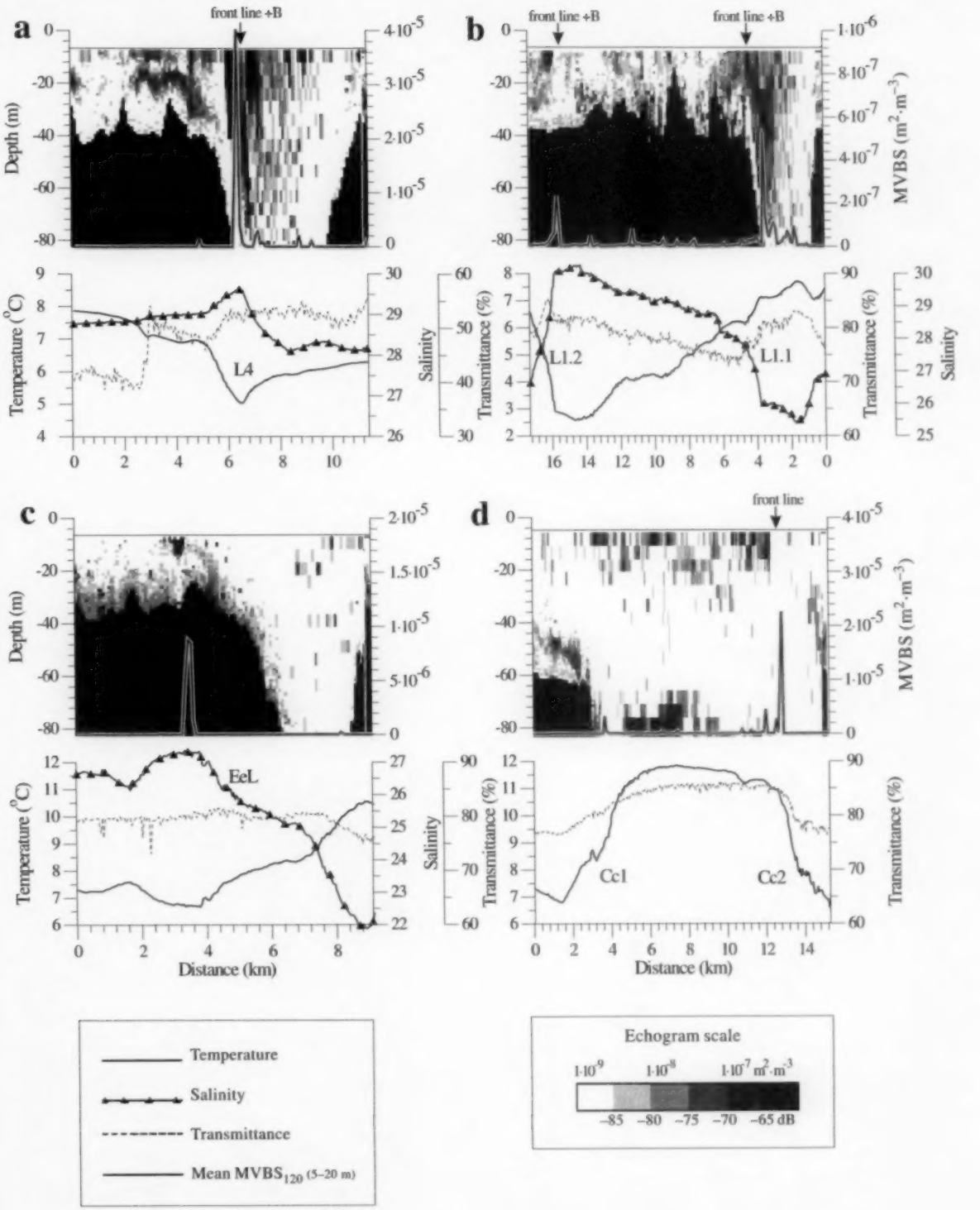
Mesoscale tidal variability at an upwelling site

Our physical data show good agreement with the frontal systems described in Ingram (1985). This complex situation is summarized below (Fig. 2). An upwelling of cold (and saltier) water from the CIL occurs during flood tide at the head of the Laurentian Channel, near the north shore (temperature and salinity panels, Fig. 7). As the flood tide progresses, the CIL is pushed upstream and covers almost the full breadth of the estuary. This can be seen in Fig. 7 from the upward and southward progression of the 4, 2, and 0°C isotherms. As the tide ebbs, the Saguenay and upper estuary waters, pushed upstream by the flood, now flow over the cold waters, which are pushed downstream and downwards. The locations of the F1, F2, and F3 transects were chosen with the aim of sampling the core of the coldest upwelled waters and its frontal boundary.

On August 10, 1995, a day before full spring tide, intense upwelling was observed in this small study area. The surface waters were cold (4.2–6.4°C) during all five surveys and generally homogeneous over the three transects in each survey (Fig. 8), except survey 1, when an intrusion of colder, more saline water was observed in the southern half of the upstream transect $F3_1$. A downstream trend in the surface temperature was observed on the three transects of survey 3 at the end of the flood.

At low tide (L) ($F1_1$–$F3_1$, Fig. 8), denser echoes in the southern portion (~60%) of all three transects indicated good longitudinal continuity of the biomass aggregation, which peaked at the water mass boundary on $F3_1$ and at the front lines on $F2_1$ and $F2b_1$ (survey 1, Figs. 7 and 8). There, the front corresponded to a sudden 20-m rise of the 4°C isotherm and the 29 isohaline. During the second survey (L + 1 to L + 2) ($F1_2$–$F3_2$, Fig. 8), a general cooling occurred in the subsurface waters all along $F2b_2$ due to a rising of the CIL waters as delimited by the 2°C isotherm or the 31 isohaline (L + 3) ($F2b_2$, Fig. 7). The most striking difference was the disappearance of the echo aggregations in the southern half

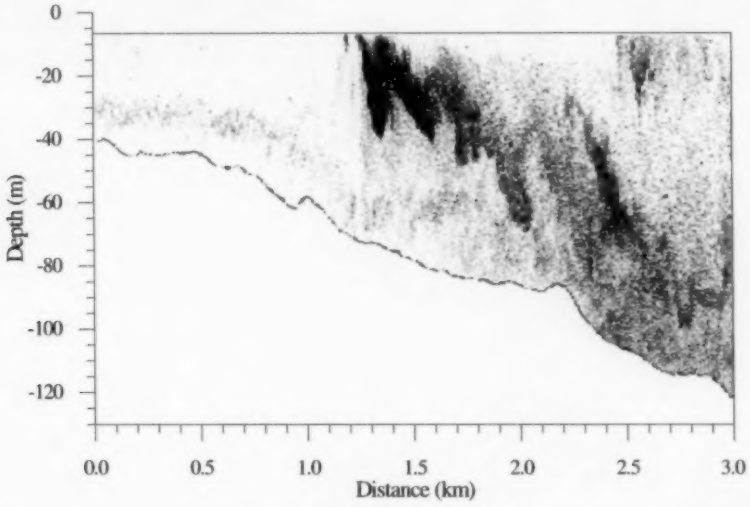
© 1999 NRC Canada



© 1999 NRC Canada

Fig. 7. Echogram of the MVBS, $MVBS_{5-20}$, and 0- to 30-m vertical structure of temperature, salinity, OPC transmittance, and particle abundance for equivalent spherical diameter classes of 1–4 and 4–7 mm along the F2b transects for the five tow-yo surveys over a tidal cycle on August 10, 1995. Surface front lines are indicated (with rorquals (+R) and seabirds (+B) when present). Left is the southeast side (i.e., Île Rouge). Survey times: $F2b_1$, 9:08–9:50, L + 1; $F2b_2$, 11:30–12:10, L + 3; $F2b_3$, 13:49–14:23, L + 6; $F2b_4$, 15:30–16:04, H + 2; $F2b_5$, 17:09–17:48, H + 3. Scales and conclusion of Fig. 7 on p. 1842.

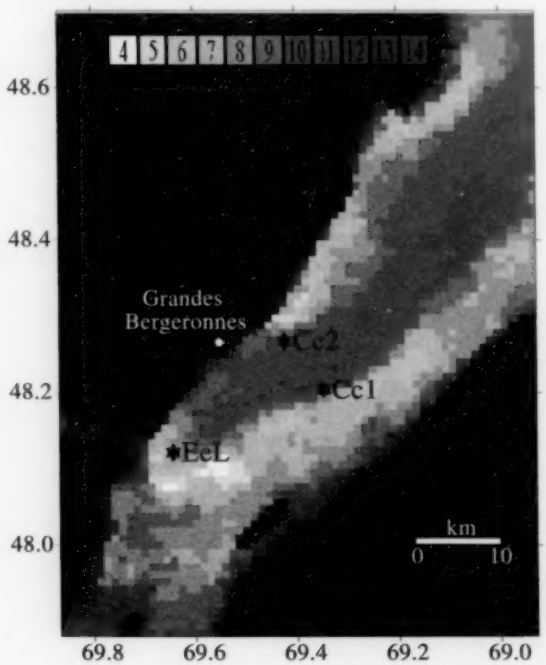
Fig. 5. High-resolution echogram of front L4 (Figs. 1*d* and 4*a*) detected near the mouth of the Saguenay Fjord on August 16, 1995, 5 h after low tide (L + 5).



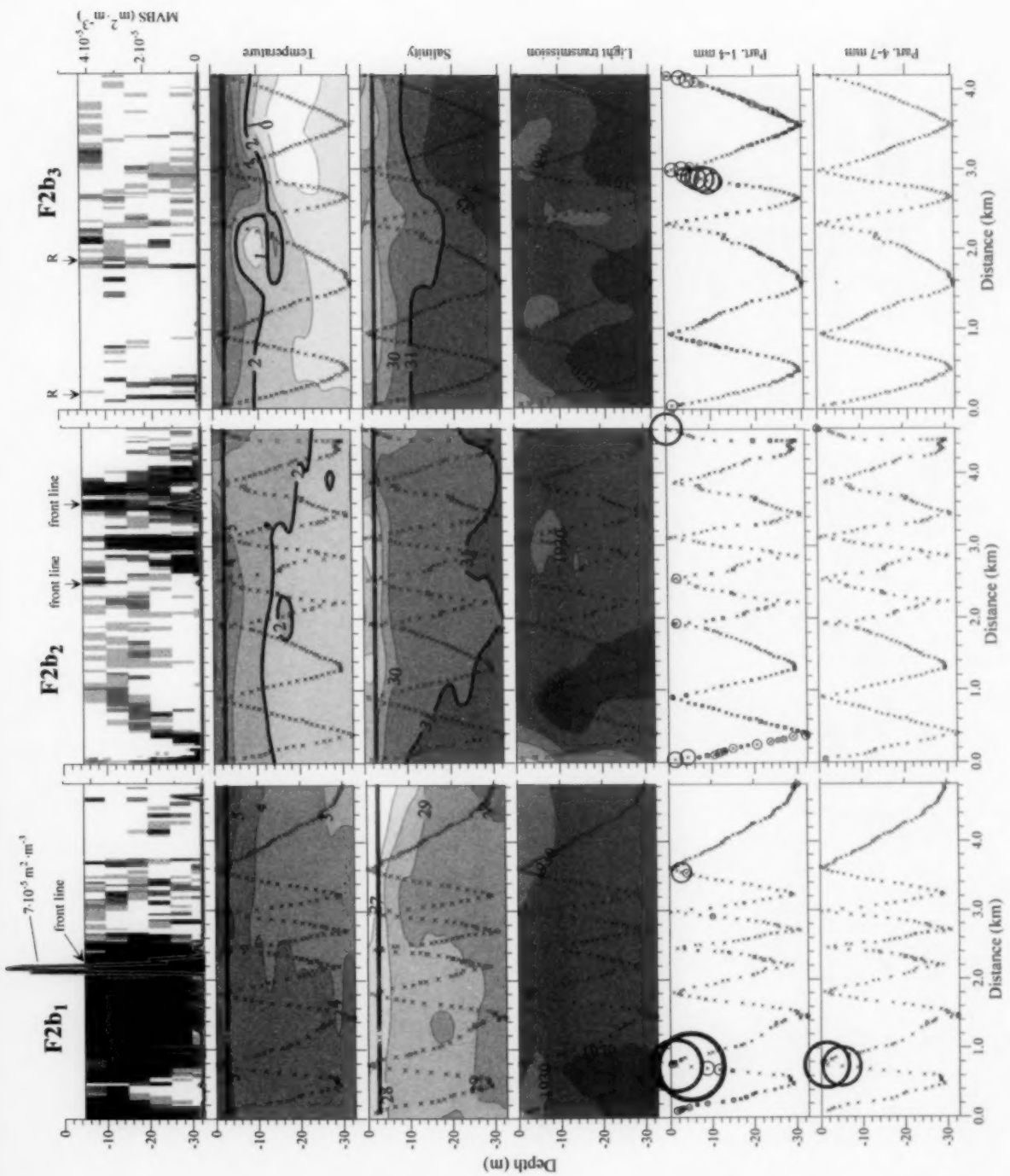
of the three transects. Small peaks of $MVBS_{5-20}$ were noted at midtransect on $F1_2$ and $F2_2$; they corresponded to a front line on $F2b_2$. The CIL continued to rise toward the surface on the third (L + 4 to L + 6) and fourth (high tide (H) to H + 2) surveys (Figs. 7 and 8). It contained a cold core with a temperature below 0°C and more transparent waters (Fig. 7). The temperature gradient indicated penetration of the CIL from the northern side. The echoes remained scattered but showed signs of forming a longitudinal aggregation between $F1_4$ and $F2_4$ along the frontal line (Fig. 8). During the fifth survey (H + 2 to H + 3) (Figs. 7 and 8), very few echoes were observed on $F1_5$ and $F2_5$, but significant biomass was reappearing on the upstream transect ($F3_5$). Concentrations were larger at the front line, where bird aggregations and rorquals were observed. The same frontal conditions and dense fish aggregations were noted on the next passage along the middle transect ($F2b_5$, Fig. 7), in contrast with the conditions observed about 50 min earlier ($F2_5$, Fig. 8). The 0- to 30-m water layer was warmer, as in survey 1, and the CIL had dropped down by 15–20 m. Surface transmittance was low at that time along all three transects but showed a rising trend in the downstream direction.

During all five surveys, the OPC data did not show a marked structure for either size-class considered (Fig. 7). No relationship was found between zooplankton density and the physical structures or the echo aggregations. Local patches, particularly for the smallest particles, were observed on the first three surveys in the warmer waters of the upper 10 m, and one patch was present on survey 5 in the cold water of the 20- to 30-m layer. The mesozooplankton composition found along the Bongo 0- to 30-m tow-yo path was domi-

Fig. 6. NOAA satellite thermal image of the LSLE showing the cold-water upwelling (light areas) and the temperature discontinuities at the head of the Laurentian Channel on August 8, 1995, at 11:07 and the localization of the fronts of Figs. 4*b* and 4*c*.



© 1999 NRC Canada



© 1999 NRC Canada

© 1999 NRC Canada

nated by the copepod *Calanus finmarchicus*, late copepodites and adults, with low mean concentrations recorded on all five surveys (Table 1).

The MVBS distribution relative to temperature (Fig. 9) for all five surveys on F2b differed from the distribution expected under the null hypothesis, which states that the distribution will not differ from random occurrence relative to temperature availability (χ^2, $p < 0.001$). It showed that most of the biomass detected in the top 30 m was in the warmest waters. Nearly 90% of the total biomass was observed in waters with temperatures above 2°C, and more than 60% was associated with water temperatures above 4°C.

Discussion and conclusion

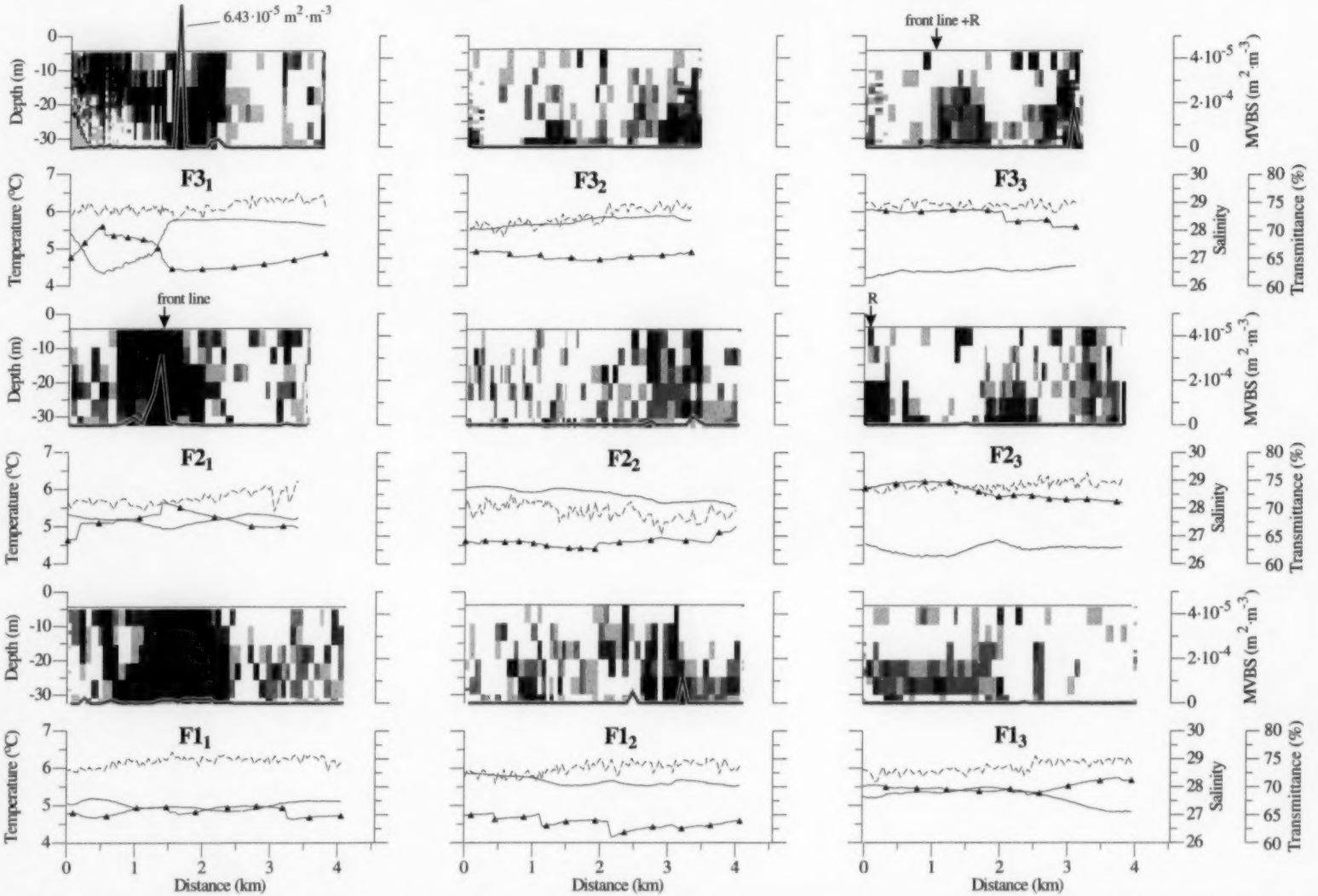
Many fronts associated with fish aggregations were regularly observed in the upstream portion of the LSLE, particularly in the shallow waters (<100 m) bordering the Laurentian Channel and especially at its head between the mouth of the Saguenay and Île Rouge. The fronts can be located on the synoptic satellite images of surface temperature (Fig. 6) (Gratton et al. 1988; Lavoie et al. 1996). Frontal structures existed at all tidal phases, but their intensity and occurrence in the different areas were probably modulated by the fortnightly cycle. On the sill at the head of the Laurentian Channel, the bottom topography coupled with tidal forcing plays a major role in the formation and location of such fronts (Ingram 1985), which occur at the point of contact between the warm surface waters and the cold upwelled waters from the CIL. At the head of the Laurentian Channel at low tide, the CIL (2°C isotherm) is usually located deeper than 30 m below the surface (Therriault and Lacroix 1976). On August 10, 1995, 1 h after high tide, the CIL was detectable at depths of 4–5 m on transect F2b. This is consistent with the findings of Therriault and Lacroix (1976), who reported that the CIL extended to depths of less than 8 m at high tide at a station downstream of this transect. During the ebb, the upwelled CIL is subsequently entrained with the upper estuary waters by the coastal jet along the south shore of the LSLE (Gratton et al. 1988; Mertz et al. 1988). The location of this jet at its origin is delimited by the cold waters in Fig. 6. The fronts noted along the south edge of the Laurentian Channel correspond to the boundary between this southern coastal jet and the warm, more saline waters originating from the northern downstream portion of the LSLE (Fig. 6). The fronts observed along the north shore of the LSLE in August 1995 appear to be associated with upwelling of the CIL due to the simultaneous action of the tide and intermittent westerly winds (Ekman transport, Lavoie et al. 1996). This system gives rise to cold filaments on both sides of the Laurentian Channel, with a contrasting mass of warm surface water confined in the middle of the estuary. Satellite thermal imagery of the area shows this regularly (Lavoie et al. 1996).

During the high-resolution study of tidal upwelling of the CIL, more biomass was associated with the warm waters, and biomass was almost always concentrated to a greater extent in the frontal area when such structures were present. What was causing the fish to concentrate there? The concentration of capelin in fronts could be linked to the higher concentrations of their food there. However, our results did not provide any support for this foraging hypothesis. First, capelin caught in the upper 40 m did not show higher stomach contents when captured in frontal areas (<1 km from the front). Second, their prey items did not concentrate in the frontal area at any phase of the tidal cycle, as revealed by the OPC measurements and the corresponding mean zooplankton biomass from the net samples. The small zooplankton patches noted in the upper 30 m did not correspond to either capelin aggregations or physical features associated with the upwelling of the CIL. Third, the frontal dynamics could not generate high concentrations of capelin prey items at the front because only low zooplankton densities were present in the surrounding shallow source areas. This was revealed by the Bongo samples collected from the stations outside the deep Laurentian Channel, which showed either very low densities or an absence of capelin prey. Therefore, a direct trophic relationship based on prey density cannot explain the aggregation of fish in the vicinity of the CIL. Besides prey density, microturbulence in frontal systems could enhance predator–prey encounter rates to some extent (Kiorboe and Saiz 1995). However, this would not compensate for the low prey density observed with both the Bongo tows in the frontal region ($<30 \cdot m^{-3}$) and the higher-resolution OPC measurements. The trophic advantage to capelin of staying at the head of the Laurentian Channel likely involves physical and biological processes occurring at scales larger than those addressed in this paper, notably the three-dimensional tidal advection of rich Laurentian Channel waters, combined with the zooplankton diel vertical migration and (or) mesoscale movements of fish.

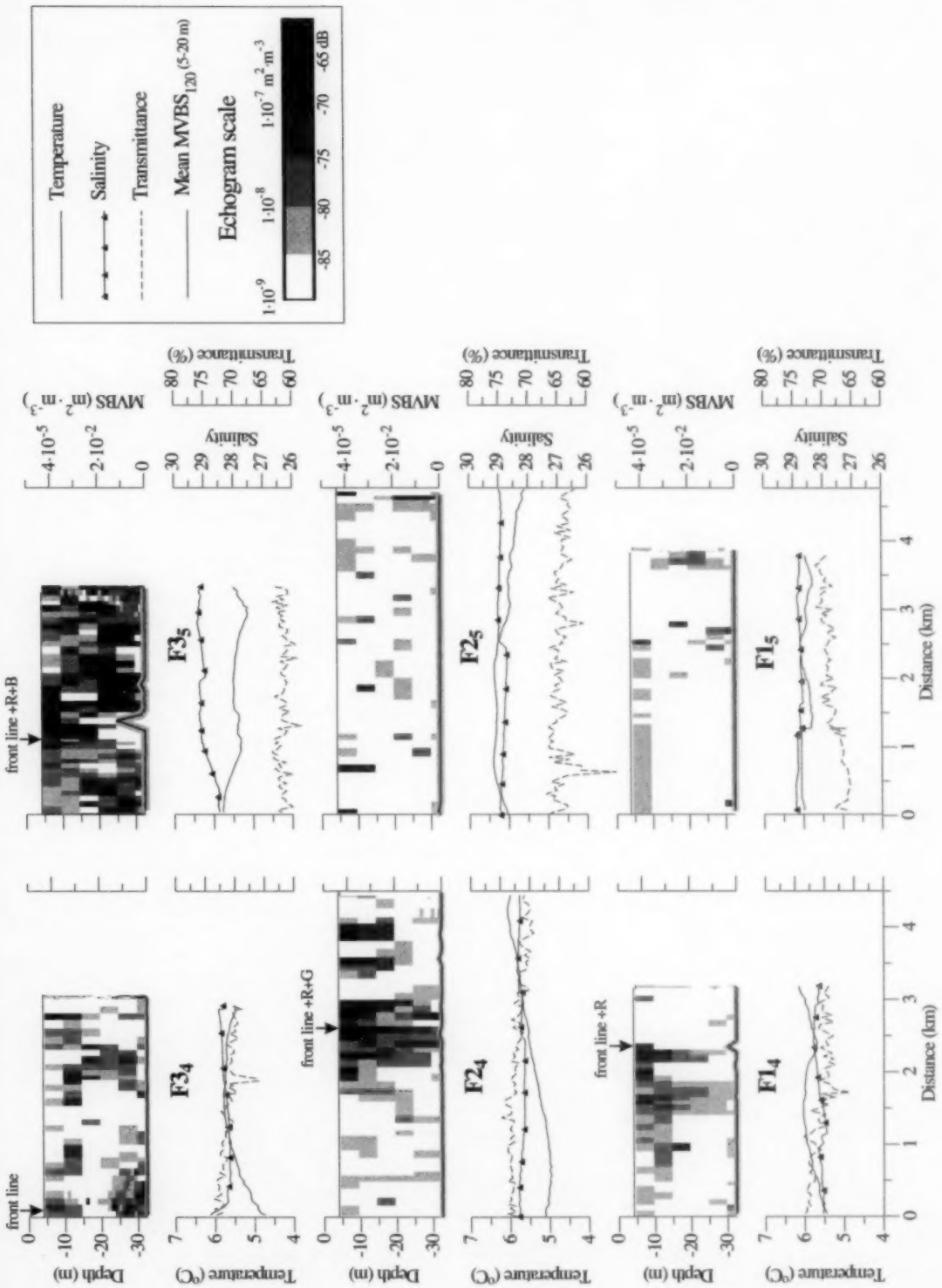
All of the above findings support our initial hypothesis that capelin aggregate in fronts in reaction to sudden temperature changes in their immediate environment. Capelin are sensitive to the sharp temperature variations caused by occasional strong events of wind-driven upwelling (Schneider and Methven 1988; Schneider 1994; Ings et al. 1997). Our results indicate that capelin also respond to the more rapid temperature changes caused by tidally driven upwellings. Capelin that occupy the upper water column at the head of the Laurentian Channel are most likely acclimated to the 2–5°C temperature range that characterizes the surface water layer. When the CIL upwells in the area during flood tide, fish seem to avoid the cold water (<2°C) and, instead, remain in the warmer waters bordering the CIL, to which they are acclimated. First, the thinning of the warm surface layer caused by the cold-water upwelling may cause the capelin to concentrate near the surface in the warmer water, especially at the rising water mass interface. When fish approach the surface, they may swim downwards to reduce exposure to visual predators (Appenzeller and Leggett 1995), possibly enhancing the aggregation process. Second, when the CIL reaches the surface, fish probably avoid these cold waters and swim away from the CIL in a horizontal direction. According to Ingram (1985), these fronts propagate at a speed of about 40 $cm \cdot s^{-1}$. This corresponds to a swimming speed of three to four body lengths per second, which a typical capelin can sustain for prolonged periods (Videler 1993). Such behaviour allows fish to remain within their acclimated temperatures and cope with the rapid temperature changes that occur in this physically dynamic environment. The fish likely behave that way in most of the fronts resulting from

© 1999 NRC Canada

Fig. 8. Echogram of the MVBS, MVBS5–20 and temperature, salinity, and transmittance at -2.5 m along the three transects for the five surveys (the subscript digit indicates the corresponding survey (1–5)) over a tidal cycle on August 10, 1995. Front lines are indicated (with rorquals (+R) and seabirds (+B) when present). F2 flipped horizontally, so all panels show the southeast side (i.e., Île Rouge) on the left, irrespective of the survey direction. Survey times: F11–F31, 7:56–8:56, L; F12–F32, 10:04–11:12, L + 1 to L + 2; F13–F33, 12:20–13:17, L + 4 to L + 5; F14–F34, 14:34–15:22, H to H + 1; F15–F35, 16:12–17:06, H + 2 to H + 3.

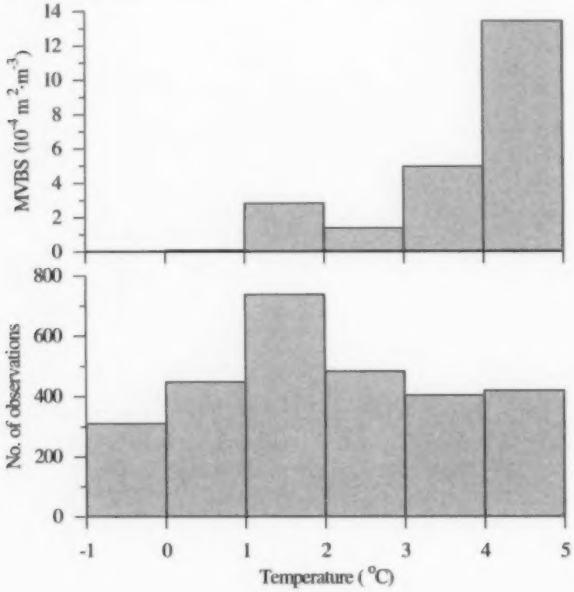


© 1999 NRC Canada



© 1999 NRC Canada

Fig. 9. Distribution of MVBS against temperature in the 5- to 30-m layer during the high-resolution surveys of transect F2b on August 10, 1995.



cold-water upwellings at the head of the Laurentian Channel. Large fish aggregations often form there, reaching concentration levels that are an order of magnitude larger than the densities found in surrounding areas. This is probably the reason for the large schools of capelin that Laurin (1982) previously reported at the edge of the Saguenay plume, one of the primary sectors of front occurrence in our study area. Immature capelin were observed to react to a temperature lower limit of around 2°C in the LSLE. Although fish can tolerate a wide range of temperatures, individuals are acclimated to a narrower temperature range (Crawshaw 1977) in a given area and time period. Reacclimation to other ranges is possible, but it is a slow process that entails bioenergetic costs (Claireaux et al. 1995). When fish are exposed to temperature gradients, they tend to concentrate within narrow thermal zones by changing their spatial distribution pattern. They display behavioural thermoregulation through preference and avoidance responses. However, capelin concentrating along the edge of the CIL might occasionally enter the CIL briefly (for a few minutes) in search of food and then return to the warmer waters that they normally inhabit. These brief sorties would not require metabolic acclimation.

Capelin are an important dietary item for many species of marine mammals and seabirds (summarized in Bailey et al. 1977; Vilhjálmsson 1994). In the North Atlantic, the species is an important food resource for minke and fin whales (Mitchell 1975; Piatt and Methven 1992), whose distributions seem to be closely linked to capelin abundance (Whitehead and Carscadden 1985; Piatt et al. 1989). Capelin is also one of the most important prey species for the resident beluga population at the head of the Laurentian Channel (Vladykov 1946; Laurin 1982). Our study shows that at the head of the Laurentian Channel, the location and timing of some capelin aggregations in the upper water column are predictable. For predators, there is a high probability of detecting dense capelin concentrations in frontal areas at such times, although capelin are also present elsewhere at the head of the Laurentian Channel (Ménard 1998). Many whales were observed close to front lines and often swimming on a parallel course. Frontal systems and upwellings are known to affect the distribution patterns of whales (e.g., finbacks, Viale 1991; minke whales, Gaskin 1982; beluga, Laurin 1982) and other predators, such as seabirds (Mehlum et al. 1998). These dynamic structures probably affect predator distribution primarily by influencing the distribution of their prey.

We have provided a systematic description of the spatiotemporal structure of fronts at the mouth of the Saguenay Fjord, in the vicinity of Île Rouge, together with capelin distribution. Our observations indicate that capelin often concentrate in these frontal regions in response to rapid temperature changes associated with tidal upwelling. These frontal aggregations were temperature mediated rather than foraging mediated. Fronts formed by the tidally driven upwelling of the CIL create a temporary physical barrier where immature capelin concentrate to avoid the cold waters. These predictable concentrations of fish in the vicinity of fronts may attract animals from higher trophic levels, providing an increased opportunity to locate adequate prey concentrations. Whales and seabirds may capitalize on these predictable barriers by developing strategies of capture that optimize their feeding success. Meanwhile, capelin, which show an active response to the barriers, are probably easier to catch. The predictable aggregations of capelin in fronts may help to explain the enduring interest that large whales have shown for the head of the Laurentian Channel. Satellite images would be useful for predicting the location of such mesoscale fronts between water masses, given the (cold) signature of the CIL. The 1-km^2 resolution would be sufficient for predicting the probable locations of large fish concentrations that could attract predators.

Acknowledgments

We wish to express our gratitude to R. Bailey, J. Benoît, M. Boulé, S. Lévesque, N. Ménard, Y. Samson, D. Thibeault, and the crews of the CSS *Creed*, *Calanus II*, and *Parizeau* for their assistance with data collection or their analysis. We are grateful to P. Larouche and B. Pettigrew for supplying the satellite thermal images. Special recognition goes to R. Bailey, M. Castonguay, M.O. Hammill, and anonymous reviewers for their helpful comments on earlier versions of the manuscript. This work is part of St. Lawrence Vision 2000 research project No. IML 113-94-9.

References

Appenzeller, A.R., and Leggett, W.C. 1995. An evaluation of light-mediated vertical migration of fish based on hydroacoustic analysis of the diel vertical movements of rainbow smelt (*Osmerus mordax*). Can J. Fish. Aquat. Sci. **52**: 504–511.

Bailey, R.F.J., Able, K.W., and Leggett, W.C. 1977. Seasonal and vertical distribution and growth of juvenile and adult capelin (*Mallotus villosus*) in the St. Lawrence estuary and western Gulf of St. Lawrence. J. Fish. Res. Board Can. **34**: 2030–2040.

© 1999 NRC Canada

Burczynski, J. 1982. Introduction to the use of sonar systems for estimating fish biomass. FAO Fish. Tech. Pap. No. 191 (Rev. 1).

Canadian Hydrographic Service. 1997. Atlas of tidal currents: St. Lawrence estuary from Cap de Bon-Désir to Trois-Rivières. Department of Fisheries and Oceans, Canadian Hydrographic Service, Ottawa, Ont.

Claireaux, G., Webber, D.M., Kerr, S.R., and Boutilier, R.G. 1995. Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. J. Exp. Biol. **198**: 49–60.

Crawshaw, L.I. 1977. Physiological and behavioral reactions of fishes to temperature change. J. Fish. Res. Board Can. **34**: 730–734.

Dommasnes, A., and Røttingen, I. 1985. Acoustic stock measurements of the Barents Sea capelin 1972–1984. A review. *In* Proceedings of the Soviet–Norwegian Symposium on the Barents Sea Capelin. *Edited by* H. Gjøsæter. Institute of Marine Research, Bergen, Norway. pp. 45–108.

Edds, P.L., and MacFarlane, J.A. 1987. Occurrence and general behavior of balaenopterid cetaceans summering in the St. Lawrence Estuary, Canada. Can. J. Zool. **65**: 1363–1376.

Focal Technologies Inc. 1992. LabWindows® OPC data acquisition software. User manual 216-0402-09 A for OPC–DAS, version 216-0403-09 A. Focal Technologies Inc., Halifax, N.S.

Foote, K.G., Knudsen, H.P., Vestnes, G., MacLennan, D.N., and Simmonds, E.J. 1987. Calibration of acoustic instruments for fish density estimation: a practical guide. ICES Coop. Res. Rep. No. 144.

Gaskin, D.E. 1982. The ecology of whales and dolphins. Heinemann, London, U.K.

Gratton, Y., Mertz, G., and Gagné, J.A. 1988. Satellite observations of tidal upwelling and mixing in the St. Lawrence estuary. J. Geophys. Res. **93**: 6947–6954.

Herman, A.W. 1992. Design and calibration of a new optical plankton counter capable of sizing small zooplankton. Deep-Sea Res. **39**: 395–415.

Herman, A.W., Cochrane, N.A., and Sameoto, D.D. 1993. Detection and abundance estimation of euphausiids using an optical plankton counter. Mar. Ecol. Prog. Ser. **94**: 165–173.

Horne, J.K. 1994. Spatial variance of capelin (*Mallotus villosus*) in coastal Newfoundland waters. J. Northwest Atl. Fish. Sci. **17**: 33–43.

Ingram, R.G. 1976. Characteristics of a tide-induced estuarine front. J. Geophys. Res. **81**: 1951–1959.

Ingram, R.G. 1985. Frontal characteristics at the head of the Laurentian trough. Nat. Can. **112**: 31–38.

Ings, D.W., Horne, J.K., and Schneider, D.C. 1997. Influence of episodic upwelling on capelin, *Mallotus villosus*, and Atlantic cod, *Gadus morhua*, catches in Newfoundland coastal waters. Fish. Oceanogr. **6**: 41–48.

Johannesson, K.A., and Mitson, R.B. 1983. Fisheries acoustics. A practical manual for aquatic biomass estimation. FAO Fish. Tech. Pap. No. 240.

Kiorboe, T., and Saiz, E. 1995. Planktivorous feeding in calm and turbulent environments, with emphasis on copepods. Mar. Ecol. Prog. Ser. **122**: 135–145.

Laevastu, T., and Hayes, M.L. 1981. Fisheries oceanography and ecology. Fishing News Books Ltd., Farnham, Surrey, U.K.

Laurin, J. 1982. Étude écologique et éthologique de la population de bélugas "*Delphinapterus leucas*" du fjord du Saguenay, Québec. M.Sc. thesis, Université de Montréal, Montréal, Qué.

Lavoie, D., Simard, Y., Benoît, J., Larouche, P., and Thibeault, B. 1996. Distribution des masses d'eau à la tête du chenal Laurentien dans l'estuaire du Saint-Laurent aux étés 1994 et 1995. Rapp. Tech. Can. Hydrogr. Sci. Océaniques No. 176.

Legendre, P., and Legendre, L. 1998. Numerical ecology. Elsevier, New York.

Mann, K.H., and Lasier, J.R.N. 1991. Dynamics of marine ecosystems: biological–physical interactions in the oceans. Blackwell Scientific Publications, Oxford, U.K.

Marchand, C. 1997. Étude des agrégations de proies potentielles pour les rorquals détectées par acoustique dans les fronts la tête du chenal Laurentien (estuaire maritime du Saint-Laurent, Québec). M.Sc. thesis, Université du Québec à Rimouski, Rimouski, Qué.

Mehlum, F., Hunt, G.L., Decker, M.B., and Nordlund, N. 1998. Hydrographic features, cetaceans and the foraging of thick-billed murres and other marine birds in the northwestern Barents Sea. Arctic, **51**: 243–252.

Ménard, N. 1998. Répartition spatiale et structure des bancs de poissons pélagiques dans une aire d'alimentation des cétacés de l'estuaire du Saint-Laurent. M.Sc. thesis, Université Laval, Québec, Qué.

Mertz, G., El-Sabh, M.I., Proulx, D., and Condal, A.R. 1988. Instability of a buoyancy-driven coastal jet: the Gaspé Current and its St. Lawrence precursor. J. Geophys. Res. **93**: 6885–6893.

Methven, D.A., and Piatt, J.F. 1991. Seasonal abundance and vertical distribution of capelin (*Mallotus villosus*) in relation to water temperature at a coastal site off eastern Newfoundland. ICES J. Mar. Sci. **48**: 187–193.

Mitchell, E.D. 1975. Trophic relationship and competition for food in northwest Atlantic whales. *In* Proceedings of the Canadian Society of Zoologists Annual Meeting, 1974. *Edited by* M.D.B. Burt. Canadian Society of Zoologists, Ottawa, Ont. pp. 123–132.

Nash, R.D.M., Magnuson, J.J., Stanton, T.K., and Clay, C.S. 1989. Distribution of peaks of 70 kHz acoustic scattering in relation to depth and temperature during day and night at the edge of the Gulf Stream-EchoFront 83. Deep-Sea Res. **36**: 587–596.

Nemoto, T. 1970. Feeding pattern of baleen whales in the ocean. *In* Marine food chains. *Edited by* J.H. Steele. University of California Press, Berkeley, Calif. pp. 241–252.

Olson, D.B., and Backus, R.H. 1985. The concentrating of organisms at fronts: a cold-water fish and warm-core Gulf Stream ring. J. Mar. Res. **43**: 113–137.

Piatt, J.F., and Methven, D.A. 1992. Threshold foraging behavior of baleen whales. Mar. Ecol. Prog. Ser. **84**: 205–210.

Piatt, J.F., Methven, D.A., Burger, A.E., McLagan, R.L., Mercer, V., and Creelman, E. 1989. Baleen whales and their prey in a coastal environment. Can. J. Zool. **67**: 1523–1530.

Rose, G.A., and Leggett, W.C. 1989. Interactive effects of geophysically-forced sea temperatures and prey abundance on mesoscale coastal distributions of a marine predator, Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **46**: 1904–1913.

Schneider, D.C. 1994. Distribution of capelin (*Mallotus villosus*) in relation to coastal upwelling in the Avalon Channel. J. Northwest Atl. Fish. Sci. **17**: 23–31.

Schneider, D.C., and Methven, D.A. 1988. Response of capelin to wind-induced thermal events in the southern Labrador Current. J. Mar. Res. **46**: 105–118.

Simard, Y., and Lavoie, D. 1999. The rich krill aggregation of the Saguenay – St. Lawrence Marine Park: hydroacoustic and geostatistical biomass estimates, structure, variability, and significance for whales. Can. J. Fish. Aquat. Sci. **56**: 1182–1197.

Simard, Y., De Ladurantaye, R., and Therriault, J.-C. 1986*a*. Aggregations of euphausiids along a coastal shelf in an upwelling environment. Mar. Ecol. Prog. Ser. **32**: 203–215.

Simard, Y., Lacroix, G., and Legendre, L. 1986*b*. Diel vertical migrations and nocturnal feeding of a dense coastal krill scattering

© 1999 NRC Canada

layer (*Thysanoessa raschi* and *Meganictyphanes norvegica*) in stratified surface waters. Mar. Biol. **91**: 93–105.

Simard, Y., McQuinn, I., Montminy, M., Samson, Y., Lang, C., Stevens, C., and Miller, D. 1998. CH1, Canadian hydroacoustic data analysis tool 1, user's manual (version 2.0). Can. Tech. Rep. Fish. Aquat. Sci. No. 2256.

Therriault, J.-C., and Lacroix, G. 1976. Nutrients, chlorophyll, and internal tides in the St. Lawrence estuary. J. Fish. Res. Board Can. **33**: 2447–2757.

Van Guelpen, L., Markle, D.F., and Duggan, D.J. 1982. An evaluation of accuracy, precision, and speed of several zooplankton subsampling techniques. J. Cons. Cons. Int. Explor. Mer, **40**: 226–236.

Vésin, J.P., Leggett, W.C., and Able, K.W. 1981. Feeding ecology of capelin (*Mallotus villosus*) in the estuary and western Gulf of St. Lawrence and its multispecies implications. Can. J. Fish. Aquat. Sci. **38**: 257–267.

Viale, D. 1991. Méthodes synoptiques de recherche des zones productives en mer: détection simultanée des cétacés, des fronts thermiques et des biomasses sous-jacentes. Ann. Inst. Océanogr. **67**: 49–62.

Videler, J.J. 1993. Fish swimming. Chapman and Hall, London, U.K.

Vilhjálmsson, H. 1994. The Icelandic capelin. J. Mar. Res. Inst., Reykjavik, Iceland. XIII. No. 1.

Vladykov, V.D. 1946. Études sur les mammifères marins. IV. Nourriture du marsouin blanc ou béluga (*Delphinapterus leucas*) du fleuve du Saint-Laurent. Contrib. Dép. Pêch. Qué. No. 17.

Whitehead, H., and Carscadden, J.E. 1985. Predicting inshore whale abundance — whales and capelin off the Newfoundland coast. Can. J. Fish. Aquat. Sci. **42**: 976–981.

© 1999 NRC Canada

Spawn date dependent survival and growth in the early life stages of *Hypoptychus dybowskii* (Gasterosteiformes)

Yoji Narimatsu and Hiroyuki Munehara

Abstract: The effects of spawn date on survival and growth during the embryonic, larval, and juvenile stages of a coastal fish, *Hypoptychus dybowskii* (Gasterosteiformes), were examined. Monitoring of embryos using SCUBA showed that the embryonic period decreased as the season progressed and the hatching rate was lowest for the early-spawn cohort (EC). Analysis of otolith daily growth increments indicated that EC larvae had much lower survival and growth rates than larvae of middle-spawn (MC) and late-spawn cohorts (LC), but there were no large differences in either rate among juveniles of the three cohorts. EC suffered the highest mortality rates in the embryonic and larval stages; these high rates were linked with slow development and growth, probably caused by below-optimum water temperatures. EC fish grew over a longer period and reached larger body size than MC and LC fish. These results suggest that intraannual variations in growth pattern and survival rate in the embryonic and larval stages were caused by spawn date differences, which favored the LC in the study year, but the EC might have a size-dependent advantage in survivorship and reproductive success during the postjuvenile stages.

Résumé : Les effets de la date du frai sur la survie et la croissance pendant les stades embryonnaire, larvaire et juvénile d'un poisson côtier, *Hypoptychus dybowskii* (Gasterosteiformes), ont été examinés. La surveillance des embryons par plongeur autonome a montré que la période embryonnaire diminuait à mesure que la saison avançait et que le taux d'éclosion était plus faible pour la cohorte ayant frayé plus hâtivement (CH). L'analyse de la croissance quotidienne des otolithes a montré que les larves CH présentaient un taux de survie et un taux de croissance de beaucoup inférieurs à ceux des larves des cohortes de mi-saison (CM) et de saison tardive (CT), mais qu'il n'y avait pas d'écarts importants pour ces valeurs chez les juvéniles des trois cohortes. Les individus de la CH sont ceux qui ont subi les taux de mortalité les plus élevés aux stades embryonnaire et larvaire. Ces taux élevés étaient liés à un développement lent et à une croissance faible s'expliquant sans doute par des températures de l'eau inférieures aux valeurs optimales. Les poissons CH ont eu une période de croissance plus longue et atteint une plus grande taille que ceux des groupes CM et CT. Les résultats obtenus portent à croire que les variations intra-annuelles de la croissance et de la survie aux stades embryonnaire et larvaire s'expliquent par des écarts de la date du frai qui favorisaient le groupe CT au cours de l'année de l'étude, mais le groupe CH pourrait disposer d'un avantage lié à la taille pour ce qui est de la survie et du succès de la reproduction au cours des étapes postjuvéniles.

[Traduit par la Rédaction]

Introduction

In fish populations, recruitment is believed to be affected more by survival during the early life stages than by adult spawning stock (Cushing 1975; Pepin 1991; Kohler et al. 1993; Walters and Juanes 1993). Many biotic and physical factors have been suggested to influence survival during the early life stages in both theoretical (Cushing 1975; Rice et al. 1993) and empirical studies (Crecco and Savoy 1985; Bailey et al. 1993; Dorsey et al. 1996; Ludsin and DeVries 1997). These factors vary throughout the year, particularly at high latitudes. The spawning seasons of temperate and subarctic fish often last more than several weeks (Cargnelli and Gross 1996). A prolonged spawning season can increase the possibility that individuals with different spawn dates will experience different environmental conditions at the same age.

The effects of temporal differences in environmental conditions on growth and survival have been investigated in the larval stage (Crecco and Savoy 1985; Rice et al. 1987; Wright and Bailey 1996). Comparison of hatch date between newly hatched larvae and juveniles through the analysis of daily otolith increments has demonstrated hatch date dependent survival and growth. A significant subject for survival in the early life stages has not yet been examined. No study has related spawn timing and survival variations in the early life stages. Embryos also suffer high mortality (McGurk 1986; Bailey and Houde 1989; Dorsey et al. 1996). Focusing on spawn date and determining how spawn date affects the early life stages and episodic mortality may produce information important for understanding recruitment.

Hypoptychus dybowskii (Gasterosteiformes), a common nearshore species in Hokkaido, Japan, spawns from mid-

Received October 29, 1998. Accepted July 6, 1999.
J14865

Y. Narimatsu.[1] Research Institute of North Pacific Fisheries, Faculty of Fisheries, Hokkaido University, Minato, Hakodate, Hokkaido 041-8611, Japan.
H. Munehara. Usujiri Fisheries Laboratory, Hokkaido University, Minami-kayabe, Hokkaido 041-1613, Japan.

[1]Author to whom all correspondence should be addressed.
e-mail: nari@fish.hokudai.ac.jp

© 1999 NRC Canada

April to late June. Female *H. dybowskii* produce an egg mass at each spawning. Spawning females attach egg masses onto sargassum stalks in territories established by males. Just after spawning, egg masses are arranged by the territorial males (Akagawa and Okiyama 1993). After the egg masses harden, they are no longer guarded by the spawning adults (Y. Narimatsu, unpublished data); embryonic survival through hatching thus depends on environmental conditions and predation. Larvae and juveniles remain around seaweed beds after hatching and can be aged by otolith analysis (Narimatsu and Munehara 1997); thus, it possible to perform long-term studies on the life history of this species.

In this study, we examined the effects of spawn date on survival and growth of *H. dybowskii* from the embryonic to juvenile stage. We assessed the spawning date distributions, hatching date distributions, and the relationship between hatching rate and spawn date based on observations of a spawning area. We also assessed the spawning date distribution of larvae and juveniles by aging individuals through otolith increment analysis. On the basis of these results, we compare survival, development, and growth rate among cohorts in embryonic, larval, and juvenile stages. We discuss how a prolonged reproductive season and spawn date may affect recruitment in subarctic nearshore fish populations.

Materials and methods

Study site

The survey was conducted in a coastal region at Usujiri (41°57′N, 140°58′E), Hokkaido, Japan. The sea bottom at the survey site is composed of sand and scattered rocks covered by sargassum beds. *Hypoptychus dybowskii* occurs in large numbers at the site throughout the year. Surface temperature ranged from 0.8 to 23.8°C throughout the year, from 4.3 to 13.3°C during the spawning season, and from 8.0 to 15.9°C during the hatching season in 1995 (water temperature data from Usujiri Fisheries Laboratory, Hokkaido University). The sampling site is surrounded on two sides by seaweed and on one side by a cement breakwater.

Estimates of hatching success of egg mass

We observed the territories of 11 males in a 50-m^2 area of a sargassum bed using SCUBA once about every 3 days from 20 April to 14 July 1995. All egg masses were identified by marking the branches of seaweed with colored tags. We have not investigated the effect of tags on egg mass predation. However, we believe that the effect, if any, is small because the tags are small and inconspicuous. Egg masses contained 19–83 eggs (mean ± SD = 50.0 ± 14.0, n = 103), and the number of eggs spawned in a mass did not change significantly through the spawning season (τ = 0.014, $p > 0.05$; Y. Narimatsu, unpublished data). We recorded the spawn date of each mass and the developmental stages of eggs within each mass. Embryonic development was divided into the following five stages depending on yolk color, the presence of pigmented eyes, and the density of melanophores, which can all be determined during underwater observations: stage I, the period during and after first cleavage to gastrulation (0–5 days after fertilization at 10°C); stage II, after gastrulation to the appearance of an ocular vesicle (5–8 days); stage III, after the appearance of an ocular vesicle to the appearance of melanophores on the eyes (8–18 days); stage IV, after the appearance of melanophores on the eyes to the appearance of melanophores on the body (18–21 days); stage V, after the appearance of melanophores on the body to hatching (21–27 days). Hatching success was estimated for each egg mass. Although some masses were eaten by predatory demersal fishes such as greenlings (*Hexagrammos otakii* and *H. octogrammus*) and blennies (*Opisthocentrus dybowskii* and *O. ocellatus*), none were partially eaten, and no dead eggs were observed in any masses. Stage V eggs were recorded as "hatched" and stage I–IV eggs were recorded as "eaten" if they disappeared between observations. Hatching and predation dates were recorded. Some seaweed with attached egg masses was torn from rocks near the roots after a storm. These masses were recorded as "separated" and eliminated from the analyses.

Measurement and age analysis of larvae and juveniles

Larvae and juveniles were collected near the spawning area five times at 12- to 22-day intervals from 29 June to 1 September. We caught individuals using SCUBA with two dip nets (80 and 60 cm in diameter with 0.6-mm mesh), taking care to collect all individuals within each school. Three to five schools were caught and mixed at each sampling to avoid the effects of body size segregation among the schools. This sampling method made it possible to catch representative samples on a given date without being confounded by gear selectivity and net avoidance. A total of 852–6906 individuals were caught at each sampling. All fish were transported to the Usujiri Fisheries Laboratory, Hokkaido University, and preserved in 90% ethanol. Two hundred fish were randomly selected from each sampling for analysis. Total lengths (TL) were measured to the nearest 0.1 mm with a micrometer or calipers and corrected for average shrinkage after preservation (92.5%; Y. Narimatsu, unpublished data). The corrected lengths were used as total lengths for all analysis. Sagittal otoliths were then removed for age analysis. The sagittae were washed on a glass slide, dried, and glued to a glass slide with a drop of fingernail enamel. Growth increments of this species are deposited daily and were counted one time per each sagitta according to the method described by Narimatsu and Munehara (1997). Spawn dates were estimated in two steps: by estimating hatch dates from the age of fish at the time of collection and by estimating spawn dates using the relationship between spawn dates and hatching dates observed at the spawning area.

Comparison of survival and growth rate among cohorts

To investigate differences in relative survivorship and growth rates among prepeak, at peak, and postpeak spawning periods, egg masses, larvae, and juveniles were divided into three cohorts based on spawn date: early cohort (EC, spawned before 1 May), middle cohort (MC, spawned during 1–23 May), and late cohort (LC, spawned after 23 May). Hatching rates and daily mortality rates were compared among the cohorts. The daily mortality rate (Z) was calculated as

$$Z = [\log_e(N/N_h)]t^{-1}$$

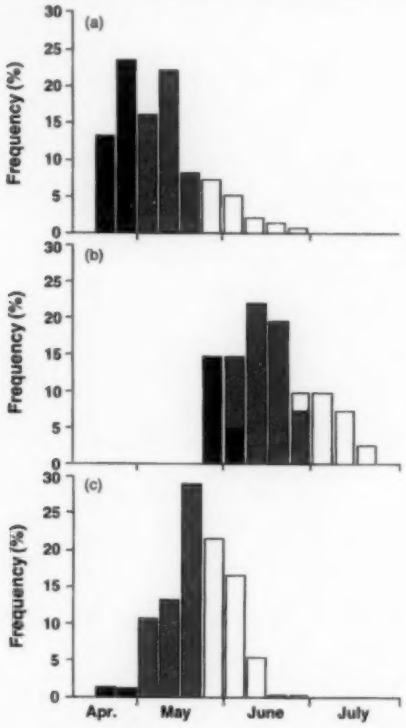
where N is the total number of spawned egg masses in each cohort, N_h is the number of hatched egg masses, and t is the mean interval in days from spawning to hatching.

Larval and juvenile growth trajectories were compared among cohorts. The timing of metamorphosis in fish is usually determined by body size rather than age (Chambers et al. 1989). *Hypoptychus dybowskii* metamorphoses to juvenile stage from 10.0 to 13.0 mm TL (Okiyama 1988), so individuals <13.0 mm TL were classified as larvae and those ≥13.0 mm TL were classified as juveniles. Growth trajectories of larval and juvenile stages among cohorts were compared using ANCOVA. Total lengths at each sampling date were compared among cohorts using ANOVA and a post hoc test.

Relative numbers at each sampling were compared to determine the growth stage when mortality rates differed. First, daily mortality rates were calculated according to the Peterson and Wroblewski (1984) model, which describes the relationship between natural mortality rate and dry weight for marine organisms. We then revised the spawn date frequency distribution taking into account

© 1999 NRC Canada

Fig. 1. Frequency distributions of spawn and hatch date of *H. dybowskii* observed in 1995. (*a*) Actual spawn date distribution observed at the spawning site, (*b*) actual hatch date distribution observed at the spawning site, and (*c*) spawn date distribution of survivors in the early life stages back-calculated from daily growth increments. Cohorts were designated as EC (spawned before 1 May) (black bars), MC (spawned during 1–23 May) (gray bars), and LC (spawned after 23 May) (white bars).



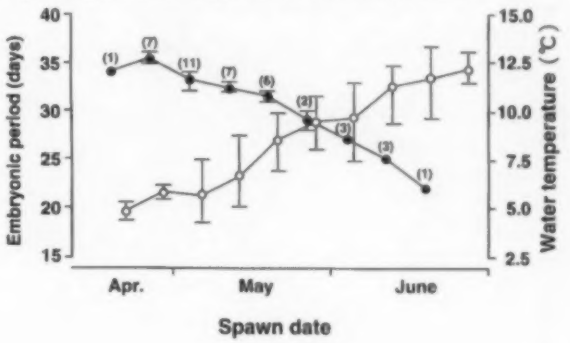
daily mortality. This allowed us to examine the representative spawn date distribution for fish at a given age. Juveniles caught on the last sampling day (1 September) were oven dried (24 h at 60°C) and weighed to the nearest 0.1 mg. Differences in hatch date distributions among samplings and in spawn date distributions between actual spawning and the last sampling day were compared using the Komogorov–Smirnov test.

Results

Hatching success

Egg masses were spawned from 20 April to 29 June 1995 (Fig. 1*a*). A total of 137 egg masses were observed in the territories of 11 males: 41 were recorded as hatched, 24 as separated, and 72 as eaten. Two seaweed stalks with attached egg masses became detached from the seafloor, probably during storms on 2 and 13 May. Eggs hatched from the fourth week of May to the third week of July (Fig. 1*b*). The EC, MC, and LC composed 28.3% (n = 32), 51.3% (n = 58), and 20.4% (n = 23) of all masses, respectively. The length of the embryonic period gradually decreased with time as the water temperature rose (Fig. 2). Hatching rates varied among cohorts, with the lowest rate occurring in the EC followed by the LC and MC. Daily mortality rates were also

Fig. 2. Relationship between spawn date and embryonic period of *H. dybowskii* off Usujiri, Japan, in 1995 (solid circles). Water temperature of the study area is also given (open circles; data from Usujiri Fisheries Laboratory, Hokkaido University, at 10:00 a.m). Circles and bars represent averages and ranges, respectively; numbers in parentheses are sample sizes.



highest for the EC embryos followed by the LC and MC (Table 1).

Relative survivorship during larval and juvenile stages

Back-calculation from daily growth increments indicated that the examined larvae and juveniles hatched from 24 May to 16 July and that 41 EC (4.1%), 482 MC (48.2%), and 477 LC fish (47.7%) survived to the sampling date. Changes in the relative frequency of each cohort at each sampling are shown in Fig. 3. Most of the LC hatched before the second sampling day. The number of LC decreased between the second and the third sampling but became stable after the third sampling. The EC composed the smallest proportion on each sampling date, suggesting that the number of EC fish decreased before the first sampling day.

Frequency distributions of hatch dates significantly differed among sample dates ($p < 0.01$), except between 16 August and 1 September ($p > 0.1$). All individuals metamorphosed into juveniles by 16 August and grew to 30 mm TL, which is about half of the adult body length (Narimatsu and Munehara 1997), by 1 September. We regarded samples caught on 1 September as survivors for the reasons described in the discussion below and adjusted the spawn date distribution (see Campana and Jones 1992).

Survivors in the early life stages had a dry weight of 24.3 ± 6.6 mg (mean ± SD). An estimated mortality rate of 1.35%/day calculated from this dry weight (Peterson and Wroblewski 1984) was used to adjust the spawn date frequency distribution. The adjusted spawn date distributions were significantly different between actual spawning observed at the spawning area and survivors in the early life stages ($p < 0.001$) (Figs. 1*a* and 1*c*). The relative frequency of the LC was 2.17 times higher (44.2%/20.4%) for survivors in the early life stages than for embryos, while the relative frequencies of the MC and EC were 1.04 times higher (53.1%/51.3%) and 10.1 times lower (2.8%/28.3%), respectively, for survivors in the early life stages than for embryos.

Difference in growth among cohorts

The relationship between the total length and the number

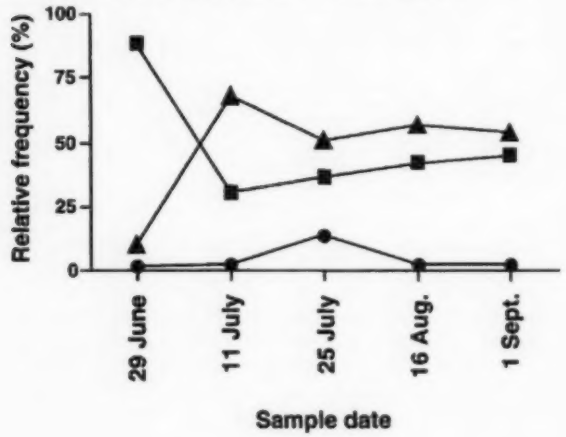
© 1999 NRC Canada

Table 1. Comparison of number, hatching rate, and daily mortality rate of egg masses among the cohorts.

Cohort	Spawn date	*n*	Separated	Eaten	Hatched	Hatching rate (%)	Daily mortality rate (%)
EC	21–30 April	51	19	24	8	25.0	3.96
MC	1–23 May	63	5	34	24	41.4	2.72
LC	4–29 June	23	0	14	9	39.1	3.45
Total		137	24	72	41	36.3	3.11

Note: Hatching rate was calculated excluding the separated egg masses.

Fig. 3. Relative frequency of EC (circles), MC (squares), and LC (triangles) larvae and juveniles for each sampling.



of daily growth increments was fit to the Gompertz growth equation as

$$L_t = 5.681\ e^{2.4131(1-e^{-0.021t})}$$

where L_t is total length (millimetres) at age t (days after hatching). Changes in growth rate at different spawn dates were examined by first combining the EC and MC to form an early–middle spawn cohort (EMC), due to the dearth of EC fish, and then comparing the EMC with the LC. The growth trajectories of the larvae and juveniles of both cohorts were described by linear regressions (Fig. 4). The LC larvae had a higher growth rate ($F_{1,460} = 121.8$, $p < 0.001$) and metamorphosed on the 21st day after hatching on average, while the EMC larvae did so on the 30th day after hatching on average. Both cohorts had statistically different ($F_{1,534} = 54.4$, $p < 0.001$) but similar growth rates after metamorphosis (EMC: 0.562 mm/day; LC: 0.516 mm/day). The earlier the cohort was spawned, the larger it was at any given time because of the longer growing time ($p < 0.05$) (Fig. 5).

Discussion

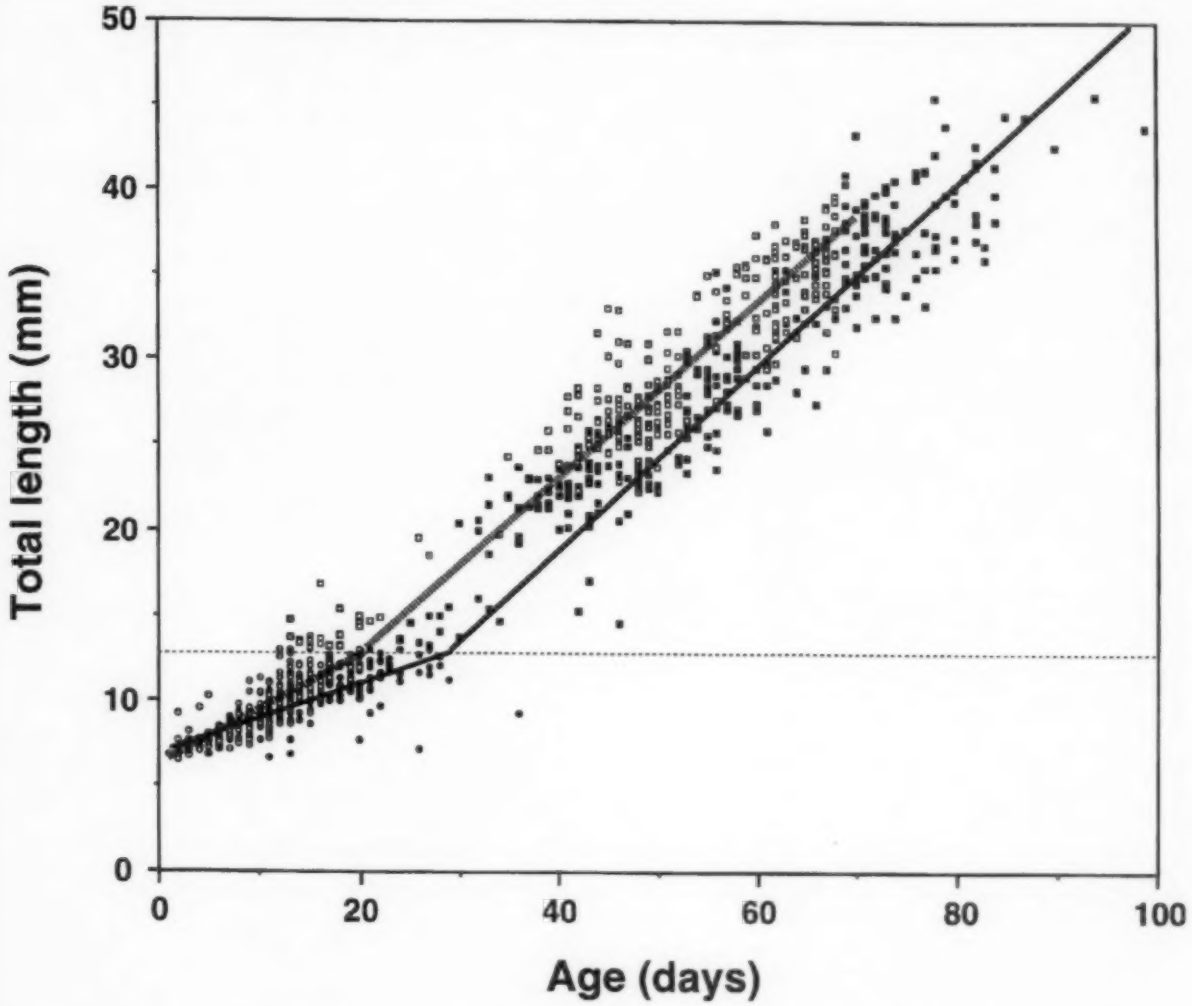
The EC, MC, and LC composed 28.3, 51.3, and 20.4% of the spawned egg masses and 19.5, 58.5, and 22.0% of the hatched embryos, respectively (Table 1). One possible explanation for the lower hatching rate of the EC embryos is related to differences in the embryonic period, which generally decreases in fishes with increasing water temperature (Alderdice and Forrester 1971; Alderdice and Velsen 1971). At the present study site, the water temperature rose from 4.3 to 13.3°C during April–June 1995, and the length of the embryonic period varied with spawn date (Fig. 2). A decrease in the embryonic period might increase the hatching success by reducing the period that embryos are susceptible to predation. A second explanation is related to differences in the daily mortality rate. Differences in the daily mortality rate among cohorts might be caused by the effect of egg mass density at the study site. Most predatory organisms that occur at the study site are visually cued predators, and hence the predation risk for an egg mass is higher when the density and abundance of egg masses are low, such as when the EC embryos were spawned.

Storms, which often occur throughout the incubating period, tore off the seaweed with egg masses. The separated egg masses probably suffer with low survival rate due to the reasons described below. The simplest reason for this is stranding. The observed spawning area lies in the subtidal zone within 100 m of the shoreline. Many of the separated egg masses become stranded along the seashore of the study site. A second possible reason is drifting away. Marliave (1986) demonstrated that larvae of several shoreline fishes resist offshore and longshore dispersal. *Hypoptychus dybowskii* live their entire lives on the shoreline. If the egg masses are transported offshore, drift away from suitable habitat could lead to high mortality. The result that embryos need at least 3 weeks to hatch also emphasizes the possibility that the separated egg masses were either stranded or transported offshore. The separated egg masses attached to seaweed composed 17.5% of all cohorts and 35.8% of the EC and 7.9% of the MC (Table 1). If the separated egg masses do not hatch at all, hatching rate of the EC and LC becomes 15.7 and 38.1%, respectively, which would contribute to an even lower survival rate of EC during the embryonic period, relative to the later cohorts.

EC embryos hatched at 8.0–11.4°C, while LC embryos hatched at 11.4–15.9°C. These different temperature ranges likely caused the lower growth rates of the EC larvae. Larval fish growth rates change with changing water temperature (Frank and Leggett 1982; Seikai et al. 1986; McCormick and Molony 1995). Lower growth rates of larvae hatched early in the reproductive season have also been reported for other fishes that breed in the spring and (or) early summer including American shad (*Alosa sapidissima*) (Crecco and Savoy 1985), bloater (*Coregonus hoyi*) (Rice et al. 1987), striped bass (*Morone saxatilis*) (Rutherford and Houde 1995), sandeel (*Ammodytes marinus*) (Wright and Bailey 1996), and largemouth bass (*Micropterus salmoides*) (Ludsin and DeVries 1997), presumably because early-hatched lar-

© 1999 NRC Canada

Fig. 4. Relationship between age and total length for EMC and LC larvae and juveniles. The horizontal broken line indicates the larvae–juvenile transition. Open circles, open squares, solid circles, and solid squares represent EMC larvae, EMC juveniles, LC larvae, and LC juveniles, respectively. Linear regressions were fitted for each growth stage of both cohorts. The solid line and broken line represent EMC and LC growth trajectories, respectively. EMC: $L_t = 0.198t + 7.058$, $r = 0.767$, $n = 229$, $p < 0.001$ (larvae); $L_t = 0.562t - 3.860$, $r = 0.985$, $n = 294$, $p < 0.001$ (juveniles). LC: $L_t = 0.319t + 6.285$, $r = 0.834$, $n = 234$, $p < 0.001$ (larvae); $L_t = 0.516t + 2.111$, $r = 0.989$, $n = 243$, $p < 0.001$ (juveniles).



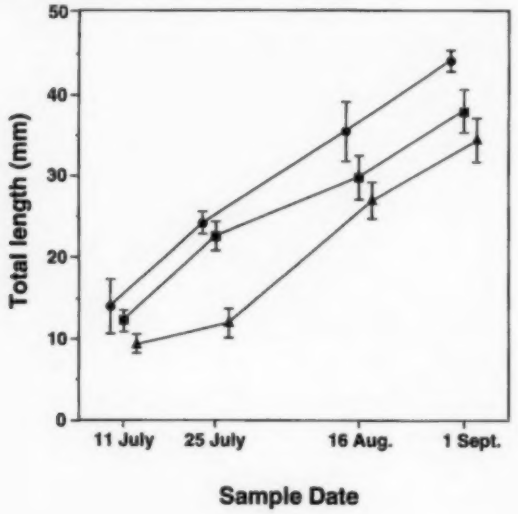
vae occur at temperatures further below their optimal temperature for growth than later-hatched larvae (Ludsin and DeVries 1997). Those spawned earlier in the season underwent longer embryonic and larval periods than those spawned later in the season.

We regarded fish caught on 1 September as survivors in the early life stages for two reasons. First, the frequency distributions of hatch dates did not significantly differ between the samples caught on 16 August and 1 September. If the mortality rates of the earliest and the latest hatched fish varied, the frequency distributions of hatch date would have differed between the samples. Second, all individuals metamorphosed into juveniles by 16 August and grew to 30 mm TL, about half as large as adults, by 1 September. This body size is generally large enough to avoid predators and to feed on prey (Miller et al. 1988; Bailey and Houde 1989).

Relative frequencies of individuals that survived through the early life stages for the EC, MC, and LC were 7.0 times lower (2.8%/19.5%), 1.1 times lower (53.1%/58.5%), and 2.0 times higher (44.2%/22.0%), respectively, than for hatching larvae of each cohort (Table 1; Fig. 1). EC fish also composed a much smaller proportion at each sampling (Fig. 3). This suggests that the number of EC fish dramatically decreased before the first sampling day (29 June), that is, during the slow-growth larval stage. EC fish contributed a larger proportion in the 25 July sample than in the other

© 1999 NRC Canada

Fig. 5. Seasonal changes in total length of *H. dybowskii* larvae and juveniles. Circles, squares, and triangles represent the EC, MC, and LC, respectively. Mean total length was significantly different at $p = 0.01$ (ANOVA and a post hoc test) among the cohorts in each sampling.



samples (Fig. 3). This may have been caused by insufficient catch size and (or) size segregation among the schools rather than by migration or mesh selection because only 852 fish in three schools were caught in this sample, while 1644–6906 fish in five schools were caught in the other samples.

Several studies have examined the hypothesis that hatch date affects the mortality rate. Larvae of American shad in the Connecticut River (Crecco and Savoy 1985), bloater in Lake Michigan (Rice et al. 1987), striped bass in Chesapeake Bay (Rutherford and Houde 1995), sandeel near Shetland island (Wright and Bailey 1996), and one of the two observed years of northern anchovy (*Engraulis mordax*) off California and Baja California (Methot 1983) that hatched early in the season exhibited higher mortality rates than those that hatched late in the season. Furthermore, most of the studies showed that early hatching is linked to slower growth, resulting in a higher mortality rate. Theoretical studies have shown that small changes in growth rates in the early life stages can affect recruitment success (Houde 1989; Pepin 1991).

Our study supports previous studies that larvae hatched early in the season grew slower and suffered high mortality rate and expands the significance of prolonged spawn season in understanding recruitment by focusing on spawn date. Relative mortality rates of *H. dybowskii* differed with growth stage; the effect of spawn date was largest in the larval stage followed by the embryonic stage and was small in the juvenile stage. The survival rates of the LC, the fastest developing and growing cohort, through the embryonic and larval stages were 1.6 and 14.0 times higher, respectively, than those of the EC, the slowest developing and growing cohort.

Survivorship in the early life stages was highest for the LC, while body size of survivors was largest in the EC. In other fishes, body size is important for overwinter survival (Post and Evans 1989; Schultz 1993; Smith and Griffith 1994; Cargnelli and Gross 1996), which will affect year-class strength (Toneys and Coble 1979; Hurst and Conover 1998). Additionally, in fish populations, larger individuals usually have a higher reproductive success than smaller ones. *Hypoptychus dybowskii* is an annual species, and most growth occurs before winter (Narimatsu and Munehara 1997). Although it has not been examined in this species, differences in body size caused by spawn date difference might affect winter survival and reproductive success.

Acknowledgments

We thank Drs. K. Shimazaki, H. Ogi, and Y. Sakurai, Faculty of Fisheries, Hokkaido University, for their valuable suggestions during this study. We express sincere thanks to Drs. Eric Tad Schultz and John Richard Bower and an anonymous referee for their helpful comments on earlier versions of the manuscript. We also thank the many people who helped us investigate and collect samples, especially the staff of the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University. Financial support for this study was provided by a research fellowship from the Japan Society for the Promotion of Science for Young Scientists and by a grant-in-aid for scientific research from the Japan Ministry of Education, Science, and Culture. This paper is contribution No. 130 from the Usujiri Fisheries Laboratory.

References

Akagawa, I., and Okiyama, M. 1993. Alternative male mating tactics in *Hypoptychus dybowskii* (Gasterosteiformes): territoriality, body size and nuptial colouration. Jpn. J. Ichthyol. **40**: 343–350.

Alderdice, D.F., and Forrester, C.R. 1971. Effect of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephlus*). J. Fish. Res. Board Can. **28**: 883–902.

Alderdice, D.F., and Velsen, F.P.J. 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). J. Fish. Res. Board Can. **28**: 1545–1562.

Bailey, K.M., and Houde, E.D. 1989. Predation on early developmental stages of marine fishes and the recruitment problem. Adv. Mar. Biol. **25**: 1–83.

Bailey, K.M., Brodeur, R.D., Merati, M., and Yoklavich, M.M. 1993. Predation on walleye pollock (*Theragra chalcogramma*) eggs and yolk-sac larvae by pelagic crustacean invertebrates in the western Gulf of Alaska. Fish. Oceanogr. **2**: 30–39.

Campana, S.E., and Jones, M. 1992. Analysis of otolith microstructure data. Can. Spec. Publ. Fish. Aquat. Sci. No. 117. pp. 73–100.

Cargnelli, L.M., and Gross, M.R. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent in a sunfish (bluegill: *Lepomis macrochirus*). Can. J. Fish. Aquat. Sci. **53**: 360–367.

Chambers, R.C., Leggett, W.C., and Brown, J.A. 1989. Egg size, female effects, and the correlations between early life history traits of capelin *Mallotus villosus*: an appraisal at the individual level. Fish. Bull. U.S. **87**: 515–523.

Crecco, V.A., and Savoy, T.F. 1985. Effects of biotic and abiotic factors on growth and relative survival of young American shad, *Alosa sapidissima*, in the Connecticut River. Can. J. Fish. Aquat. Sci. **42**: 1640–1648.

Cushing, D.H. 1975. Marine ecology and fisheries. Cambridge University Press, London, U.K.

© 1999 NRC Canada

Dorsey, S.E., Houde, E.D., and Gamble, J.C. 1996. Cohort abundances and daily variability in mortality of eggs and yolk-sac larvae of bay anchovy, *Anchoa mitchilli*, in Chesapeake Bay. Fish. Bull. U.S. **94**: 257–267.

Frank, T.K., and Leggett, W.C. 1982. Environmental regulation of growth rate, efficiency, and swimming performance in larval capelin (*Mallotus villosus*), and its application to the match/mismatch hypothesis. Can. J. Fish. Aquat. Sci. **39**: 691–699.

Houde, E.D. 1989. Subtleties and episodes in the early life of fishes. J. Fish. Biol. **35**(Suppl. A): 29–38.

Hurst, T.P., and Conover, D.O. 1998. Winter mortality of young-of-the-year Hudson river striped bass (*Morone saxatilis*): size-dependent patterns and effects on recruitment. Can. J. Fish. Aquat. Sci. **55**: 1122–1130.

Kohler, C.C., Sheehan, R.J., and Sweatman, J.J. 1993. Largemouth bass hatching success and first-winter survival in two Illinois reservoirs. N. Am. J. Fish. Manage. **13**: 125–133.

Ludsin, S.A., and DeVries, D.R. 1997. First-year recruitment of largemouth bass: the interdependency of early life stages. Ecol. Appl. **7**: 1024–1038.

Marliave, J.B. 1986. Lack of planktonic dispersal of rocky intertidal fish larvae. Trans. Am. Fish. Soc. **115**: 149–154.

McCormick, M.I., and Molony, B.W. 1995. Influence of water temperature during the larval stage on size, age and body condition of a tropical reef fish at settlement. Mar. Ecol. Prog. Ser. **118**: 59–68.

McGurk, M.D. 1986. Natural moratality of marine pelagic fish eggs and larvae: role of spatial patchiness. Mar. Ecol. Prog. Ser. **34**: 227–242.

Methot, R.D. 1983. Seasonal variation in survival of larval *Engraulis mordax* estimated from the age distribution of juveniles. Fish. Bull. U.S. **81**: 741–750.

Miller, T.J., Crowder, L.B., Rice, J.A., and Marschall, E.A. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657–1670.

Narimatsu, Y., and Munehara, H. 1997. Age determination and growth from otolith daily growth increments of *Hypoptychus dybowskii* (Gasterosteiformes). Fish. Sci. **64**: 503–508.

Okiyama, M. 1988. Hypoptychidae. *In* Atlas of the early stage fishes in Japan. *Edited by* M. Okiyama. Tokai University Press, Tokyo, Japan. (In Japanese.)

Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Can. J. Fish. Aquat. Sci. **48**: 503–518.

Peterson, I., and Wroblewski, J.S. 1984. Mortality rate of fishes in the pelagic ecosystem. Can. J. Fish. Aquat. Sci. **41**: 1117–1120.

Post, J.R., and Evans, D.O. 1989. Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. Can. J. Fish. Aquat. Sci. **46**: 1958–1968.

Rice, J.A., Crowder, L.B., and Holey, M.E. 1987. Exploration of mechanisms regulating larval survival in Lake Michigan bloater: a recruitment analysis based on characteristics of individual larvae. Trans. Am. Fish. Soc. **116**: 703–718.

Rice, J.A., Miller, T.J., Rose, K.A., Crowder, L.B., Marschall, E.A., Trebitz, A.S., and DeAngelis, D.L. 1993. Growth rate variation and larval survival inferences from an individual-based size-dependent predation model. Can. J. Fish. Aquat. Sci. **50**: 133–142.

Rutherford, E.S., and Houde, E.D. 1995. The influence of temperature on cohort-specific growth, survival, and recruitment of striped bass, *Morone saxatilis*, larvae in Chesapeake Bay. Fish. Bull. U.S. **93**: 315–332.

Schultz, E.T. 1993. The effects of birth date on fitness of female dwarf perch, *Micrometrus minimus* (Perciformes: Embiotocidae). Evolution, **47**: 520–539.

Seikai, T., Tanangonan, J.B., and Tanaka, M. 1986. Temperature influence on larval growth and metamorphosis of the Japanese flounder *Palalichthys olivaceus* in the laboratory. Bull. Jpn. Soc. Sci. Fish. **52**: 977–982.

Smith, R.W., and Griffith, J.S. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. Trans. Am. Fish. Soc. **123**: 747–756.

Toneys, M.L., and Coble, D.W. 1979. Size-related, first winter mortality of freshwater fishes. Trans. Am. Fish. Soc. **108**: 415–419.

Walters, C.J., and Juanes, F. 1993. Recruitment limitation as a consequence of natural selection for use of restricted feeding habitats and predation risk taking by juvenile fishes. Can. J. Fish. Aquat. Sci. **50**: 2058–2070.

Wright, P.J., and Bailey, M.C. 1996. Timing of hatching in *Ammodytes marinus* from Shetland waters and its significance to early growth and survivorship. Mar. Biol. **126**: 143–152.

© 1999 NRC Canada

A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations

Cecily C.Y. Chang, James Langston, Mike Riggs, Donald H. Campbell, Steven R. Silva, and Carol Kendall

Abstract: Recently, methods have been developed to analyze NO_3^- for $\delta^{15}N$ and $\delta^{18}O$, improving our ability to identify NO_3^- sources and transformations. However, none of the existing methods are suited for waters with low NO_3^- concentrations (0.7–10 μM). We describe an improved method for collecting and recovering NO_3^- on exchange columns. To overcome the lengthy collection loading times imposed by the large sample volumes (7–70 L), the sample was prefiltered (0.45 μm) with a large surface area filter. Switching to AG2X anion resin and using a coarser mesh size (100–200) than previous methods also enhanced sample flow. Placement of a cation column in front of the anion column minimized clogging of the anion column by dissolved organic carbon (DOC) accumulation. This also served to minimize transfer of unwanted oxygen atoms from DOC to the ^{18}O portion of the NO_3^- sample, thereby contaminating the sample and shifting $\delta^{18}O$. The cat-AG2X method is suited for on-site sample collection, making it possible to collect and recover NO_3^- from low ionic strength waters with modest DOC concentrations (80–800 μM), relieves the investigator of transporting large volumes of water back to the laboratory, and offers a means of sampling rain, snow, snowmelt, and stream samples from access-limited sites.

Résumé : Récemment, on a mis au point des méthodes pour déterminer les valeurs de $\delta^{15}N$ et de $\delta^{18}O$ dans les nitrates, méthodes qui permettent de mieux établir les sources et les transformations de ces derniers. Cependant, aucune des méthodes existantes ne vaut pour les eaux à faibles concentrations de NO_3^- (0,7–10 μM). Nous décrivons une méthode améliorée pour recueillir et récupérer le NO_3^- dans des colonnes échangeuses. Pour réduire les temps de chargement qui autrement seraient longs en raison de la grosseur des échantillons (7–70 L), ces derniers ont été préfiltrés (0,45 μm) avec un grand filtre. L'utilisation d'une résine anionique AG2X et d'un maillage plus grossier (100–200) que celui des autres méthodes a aussi facilité le passage des échantillons. L'installation d'une colonne cationique devant la colonne anionique a minimisé l'encrassement de la colonne anionique par accumulation de carbone organique dissous (COD). Cela a aussi permis de minimiser le transfert d'atomes d'oxygène indésirables depuis le COD vers le stock de ^{18}O de l'échantillon de nitrate, et donc une contamination de l'échantillon se soldant par une hausse de $\delta^{18}O$. Cette méthode utilisant la résine cationique et la résine AG2X permet de recueillir les échantillons sur le terrain et donc de recueillir et de récupérer le NO_3^- présent dans des eaux à faible force ionique pauvres en COD (80–800 μM), libère son utilisateur de la nécessité de transporter de grands volumes d'eau au laboratoire et permet d'échantillonner les eaux de pluie, la neige, les eaux de fonte et les eaux de ruisseau en des endroits peu accessibles.

[Traduit par la Rédaction]

Introduction

Recent publications have shown that use of both N and O isotopes of NO_3^- facilitates more confident identification of sources and processes under some conditions than when only ^{15}N is used (Böttcher et al. 1990; Aravena et al. 1993; Kendall et al. 1998; Wassenaar 1995; Böhlke et al. 1997). The main differences between the existing methods are the form of NO_3^- that the sample NO_3^- is converted to (KNO_3 versus $AgNO_3$) and the way in which the NO_3^--derived oxygen is converted to CO_2 for mass spectrometer analysis of $\delta^{18}O$.

Amberger and Schmidt (1987) first measured NO_3^- $\delta^{18}O$ using KNO_3 as a NO_3^- source and $Hg(CN)_2$ as a combustion reagent. This method, in which samples were completely desiccated without preconcentration, has been successfully used for samples with NO_3^- concentrations >40 μM (Voerkelius 1990; Durka et al. 1994). The method of Silva et

Received December 1, 1998. Accepted June 22, 1999.
J14925

C.C.Y. Chang,[1] J. Langston, S.R. Silva, and C. Kendall. U.S. Geological Survey, Water Resources Division, Mailstop 434, 345 Middlefield, Menlo Park, CA 94025, U.S.A.
M. Riggs. Department of Statistical Research, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709, U.S.A.
D.H. Campbell. U.S. Geological Survey, Water Resources Division, Federal Center, Denver, CO 80225, U.S.A.

[1]Author to whom all correspondence should be addressed. e-mail: ccchang@usgs.gov

© 1999 NRC Canada

Table 1. Water chemistry from three sites used in the anion–cation experiment.

Parameter	Hidden Villa	Ward Creek	Sacramento
K^+	25.1	1.9	66.7
Mg^{2+}	1333.8	<0.8	0.8
Ca^{2+}	159.5	<0.5	<12.5
Total Fe	3.6	<1.0	na
Mn^{2+}	<1.0	<1.0	na
Al^{3+}	5.1	0.4	na
Ba^{2+}	1.4	0.1	na
DOC	79	144	114
Sr^{2-}	5.4	na	na
Si^{4-}	310.4	1.8	na
F^-	5.3	nd	nd
Cl^-	536.3	8.5	367.9
Br^-	0.5	nd	0.9
NO_3^-	<1.4	1.4	<1.4
PO_4^{3-}	nd	nd	1.9
SO_4^{2-}	625.0	1.7	86.0
Specific conductance ($\mu S \cdot cm^{-1}$)	415	30	na

Note: Concentrations are μM; 1 ppm DOC ≈ 80 μM DOC (Qian and Mopper 1996). na, not available; nd, not determined.

al. (2000) concentrates the NO_3^- onto 2-mL anion-exchange columns, eliminating the need for water sample preservation, transport, and handling of cyanide. NO_3^- is eluted with HCl in the laboratory and converted to $AgNO_3$, and after freeze-drying, $AgNO_3$ and pure graphite are combusted in a sealed tube to produce CO_2 for $\delta^{18}O$ analysis. This approach can be used on samples with NO_3^- concentrations ranging from 20 to 40 μM (Kendall et al. 1995; Wassenaar 1995; Aravena and Robertson 1998; Harrington et al. 1998), provided that the total load of dissolved organic carbon (DOC) in the bulk sample does not exceed 1.6 mmol DOC (1 ppm DOC, ~80 μM; Qian and Mopper 1996).

Recently, Revesz et al. (1997) developed an alternative method for combusting KNO_3. Catalyzed graphite is used to produce CO_2, K_2CO_3, and N_2, and the constant oxygen isotopic fractionation between CO_2 and K_2CO_3 is used to correct the $\delta^{18}O$ values. This method was used to analyze standard reference material, groundwater, and NO_3^--rich soils from the Atacama Desert, Chile.

To date, none of these approaches use a collection method suitable for waters with low NO_3^- concentrations (0.7–20 μM). Such samples require larger sample volumes (5–70 L), and the problems resulting from DOC accumulation become more acute. When the collection protocol of Silva et al. (1999) was used, we experienced extended collection times (24 h), column clogging, and erratic CO_2 yields ranging from 30 to 300%. Low yields suggested inefficient loading due to clogging, overestimation of NO_3^- loading, or loss during sample preparation in the laboratory. Conversely, CO_2 yields >100% suggested an underestimation of sample loading or the introduction of oxygen from other oxygen-bearing compounds such as DOC. Accumulation of DOC on anion-exchange columns leads to column clogging, competition for exchange sites that would otherwise be available for NO_3^-, and the potential transfer of unwanted oxygen atoms from DOC to the CO_2 sample. While the usual approach for DOC removal has been to add activated charcoal to the bulk sample (Amberger and Schmidt 1987; Durka et al. 1994), the extent to which NO_3^- is adsorbed on the charcoal is not known. It also is possible that some of the DOC may not be removed.

In this paper, we describe an experiment designed to identify optimal methods for obtaining $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- from sites where NO_3^- concentrations are low and where specific conductivity is <450 $\mu S \cdot cm^{-1}$. Four treatment combinations were tested on water samples collected from three different sites. Additional experiments were undertaken to ensure that the method of choice was robust and to evaluate the behavior and effect of DOC on NO_3^- recovery. The objectives of these additional experiments were to determine (*i*) whether oxygen atoms from DOC are transferred to the CO_2 portion of the sample, which should contain only oxygen atoms from NO_3^-, and (*ii*) whether, if oxygen from DOC is transferred, the use of charcoal minimizes this transfer without adsorbing NO_3^-.

Materials and methods

Water chemistry

To assess the robustness of our methods, water from three sites in California with different chemical compositions was used (Table 1). Water from Ward Creek, a tributary to Lake Tahoe, was characterized by low ionic strength, low conductivity, (30 $\mu S \cdot cm^{-1}$), and a DOC concentration of 144 μM. Water from Sacramento Slough, near Knights Landing, drains rice fields east of the Sacramento River and had higher ionic strength and a DOC concentration of 224 μM. Water from Hidden Villa stream, located in the foothills of the Santa Cruz Mountains, had the highest conductivity (415 $\mu S \cdot cm^{-1}$) and a DOC concentration of 80 μM. SO_4^{2-}, which can interfere with NO_3^- adsorption on anion-exchange resin, varied in concentration by nearly three orders of magnitude among the three sites.

Anion and cation resin experiment

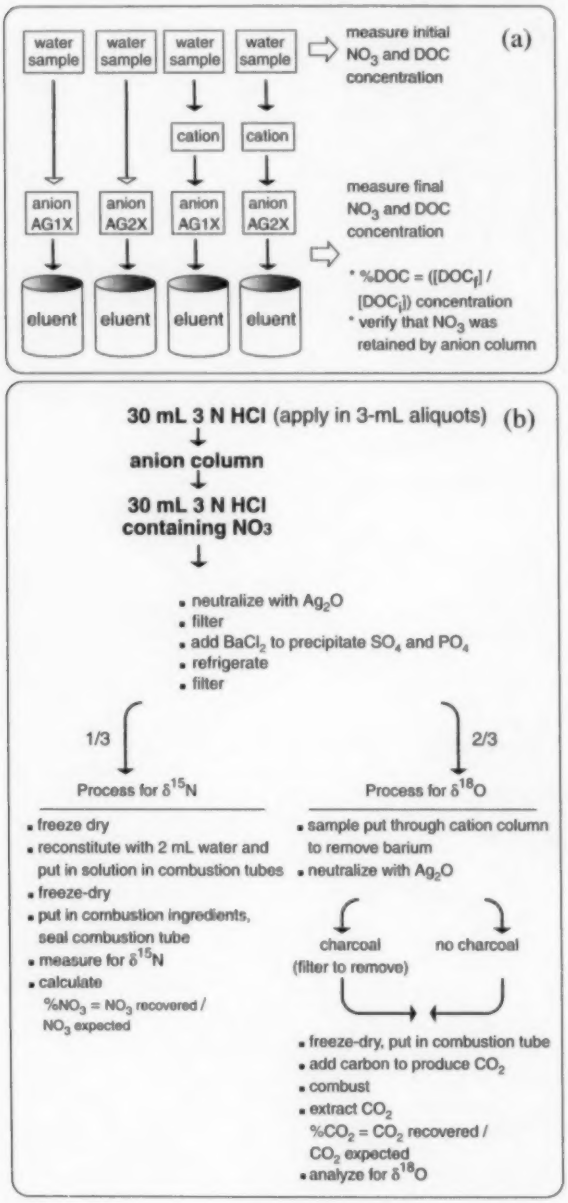
The effects of three factors on five response variables were measured. The factors were (*i*) cation-exchange resin (present or absent), (*ii*) type of anion-exchange resin (AG1X or AG2X, Biorad[2]), and (*iii*) activated charcoal added (present or absent) to the $\delta^{18}O$ portion of the sample (see next section) (Fig. 1). Samples receiving cation treatment were passed through a column filled with cation resin that was placed in front of the anion column.

The variables %DOC, $\%NO_3^-$, and $\%CO_2$ were defined as follows. %DOC was calculated as the ratio of final DOC concentration to initial DOC concentration and was used to determine whether DOC was retained by the anion column (Fig. 1*a*). Final concentration was the concentration after the sample passed through the anion column. Values of 100% indicate that no DOC was retained by the anion column. $\%NO_3^-$ was calculated as the ratio of NO_3^- recovered to expected NO_3^- recovered in the $\delta^{15}N$ portion of the sample, after processing in the laboratory. Recovery of CO_2 ($\%CO_2$) was calculated as the ratio of the amount of CO_2 recovered to the expected amount of CO_2. Expected NO_3^- and CO_2 are known because after the NO_3^- is eluted off the anion column, a known amount of sample is taken for $\delta^{15}N$ or $\delta^{18}O$ analysis (see NO_3^- elution below) (Fig.1*b*). To convert NO_3^- oxygen to CO_2,

[2]Use of brand names is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

© 1999 NRC Canada

Fig. 1. (*a*) Schematic representation of water sample collection methods for stable isotope analysis of NO_3^- and (*b*) flow diagram depicting the procedure for eluting NO_3^- from an anion column and processing for $\delta^{15}N$ and $\delta^{18}O$ (adapted from Silva et al. 2000).



$AgNO_3$ was combusted with carbon, CO_2 was extracted, and $\delta^{18}O$ was measured with a mass spectrometer.

$\delta^{18}O$ and charcoal effects

To evaluate the effect of charcoal (Norit G-60), the $\delta^{18}O$ portion of the sample was divided in half. One half was treated with activated charcoal (200 mg·L^{-1}) and the other portion was processed without charcoal. The charcoal-treated sample was shaken for 20 min and then filtered through a 47-mm-diameter nylon filter (0.2 µm). Both $\delta^{18}O$ portions were processed as described in Silva et al. (2000).

Additional experiments with the cat-AG2X method

To determine whether $BaCl_2$ affects NO_3^- recovery (see NO_3^- elution below), a KNO_3 solution was prepared using the same KNO_3 used in the cation–anion experiments ($\delta^{15}N$ = +3.49‰, $\delta^{18}O$ = +26.3‰). The KNO_3 (660 µmol) was dissolved in deionized water and passed through the cat-AG2X columns. The sample was split in half after the NO_3^- was eluted from the column. Half of the sample was processed with $BaCl_2$ and half without. From each split, two samples containing 60 µmol of NO_3^- were processed for $\delta^{15}N$ and two samples containing 100 µmol of NO_3^- were processed for $\delta^{18}O$.

To evaluate the performance of the cat-AG2X method on samples with DOC loads typical of 20 L of snow (800–3200 µmol), a solution containing the KNO_3 reagent (240 µmol) and DOC (2400 µmol) was prepared in 3 L of deionized water. These solutions are referred to as "simulated field samples." The DOC solution was made by leaching local chestnut leaves and pine and fir needles in deionized water. After the NO_3^- was eluted from the column, three 40-µmol portions were analyzed for $\delta^{15}N$ and three 30-µmol portions were analyzed for $\delta^{18}O$. Five sets of field samples were prepared. In all cases, charcoal treatment was not used for the $\delta^{18}O$ portion.

To determine the amount of DOC generated by the columns and reagents (i.e., procedural blanks), three 40-mL samples of deionized water were passed through cat-AG2X columns. The anion columns were processed with standard procedures and the DOC concentration was measured after neutralization with Ag_2O and filtering to remove barium precipitates. Comparisons were also made with and without charcoal.

To determine whether DOC is being transferred to the $\delta^{18}O$ of portion of a field sample, three 9-L samples of Sacramento water were loaded onto cat-AG2X columns. Sacramento water was used because it had the highest DOC concentration (224 µM) among our samples. The resulting total mass of DOC in 9 L is typical of the mass of DOC in a 20-L snowmelt sample. After 2 L had passed through, an aliquot of the effluent from the cation column and an aliquot of the effluent from the anion column were collected for DOC analysis. As in the above experiment, the DOC concentration was measured after removal of the barium precipitate, with and without charcoal.

Equipment

Resin columns (20-mL Econo-Pac columns, Biorad) were filled with 5 mL of either AG1X or AG2X 100–200 mesh anion-exchange resin. Cation columns were filled with 5 mL of cation-exchange resin (AG50-WX8 100–200 mesh, Biorad). While the ion-exchange capacity of all resins was 1.2 mequiv.·mL^{-1}, the relative selectivity for anions is about 10 times higher for AG1X resin (e.g., selectivity for HSO_4^{2-} and NO_3^- is 85 and 65 for AG1X resin and 15 and 8 for AG2X resin).

Three liters of water from each site was filtered through Gelman water capsules (0.45 µm), put into a collapsible container, and spiked with 250 µmol of KNO_3 with $\delta^{15}N$ = +3.49‰ and $\delta^{18}O$ = +26.3‰. Connections from the water sample container to the columns and from the anion- to the cation-exchange column were made with tubing and rubber stoppers. The stopper was secured to the column with a stainless steel joint clamp (size 35/20). The jaws on one side of the clamp were squeezed in with a pair of pliers, permitting the crimped jaws to hold the stopper in place. A small stopcock was used to maintain a maximum flow rate not exceeding 2 mL·min^{-1} and was periodically adjusted to maintain this rate as flow through the anion column slowed. To verify that all NO_3^- in the water sample was captured by the anion column, the initial

© 1999 NRC Canada

Table 2. Results of ANOVA for 2 × 2 × 3 factorial experiments on effects of anion resin type (AG1X versus AG2X), cation resin (present or absent), and site (Hidden Villa, Ward Creek, and Sacramento) on %DOC, $\%NO_3^-$ recovery, and $\delta^{15}N$.

		P		
Source	df	%DOC	$\%NO_3^-$	$\delta^{15}N$
Anion	1,24	<0.0001	0.26	0.26
Cation	1,24	0.002	<0.0001	<0.0001
Anion × cation	1,24	0.55	<0.0001	0.40
Site	2,24	<0.0001	<0.0001	<0.0001
Site × anion	2,24	0.11	0.0001	<0.0001
Site × cation	2,24	0.03	0.24	0.004
Site × anion × cation	2,24	0.20	<0.0001	0.002
Error	24	7519	551	0.0775

Note: For error terms the tabled value is the sum of squares and not a *P* value.

NO_3^- concentration was compared with the concentration after passing through the anion column.

NO_3^- elution

NO_3^- was eluted from the anion column and processed for $\delta^{15}N$ and $\delta^{18}O$ analysis as described in Silva et al. (2000), with a few modifications due to the increased volume of anion resin. Anion columns were eluted with 30 mL of 3 N HCl and the acidic solution was neutralized with 16 g of Ag_2O, which had been cleaned to remove any traces of NO_3^- (Silva et al. 2000), and then filtered to remove the AgCl (Fig. 1*b*). To precipitate any PO_4^{3-} or SO_4^{2-} in the sample (Amberger and Schmidt 1987), 4.5 mL of 1 M $BaCl_2$ was added, enough to precipitate 3000 μmol of SO_4^{2-}, the maximum mass of SO_4^{2-} that 5 mL of resin could retain. The sample was refrigerated overnight, filtered the following day to remove $BaSO_4$ and $Ba_3(PO_4)$ precipitate, and then dripped through a 9-mL cation column to remove excess barium and silver. To neutralize and convert NO_3^- to $AgNO_3$, a small amount of Ag_2O was added to the sample, after which it was filtered to remove AgCl and split into thirds, one third for $\delta^{15}N$ analysis and two thirds for $\delta^{18}O$ analysis. The $\delta^{15}N$ portion was freeze-dried, transferred into quartz combustion tubes, and processed as described in Kendall and Grim (1990).

Statistical analysis

To evaluate the variables %DOC, $\%NO_3^-$, and $\delta^{15}N$, the four treatment combinations (AG1X, AG2X, cat-AG1X, cat-AG2X) were replicated three times. Twelve samples of each type of water were randomized to each of the four combinations (Fig. 1*a*). Effects of study site, cation resin type, and anion resin type on %DOC, $\%NO_3^-$, and $\delta^{18}O$ were evaluated by a 2 × 2 × 3 ANOVA for the completely randomized design (CRD). To evaluate the variables $\%CO_2$ and $\delta^{18}O$, randomized assignment of water samples to cation resin and anion resin treatments was done as in the first experiments. Following the initial randomization, each of the samples was divided in half and each half randomly assigned to treatment with or without charcoal. The results were analyzed as a CRD split-plot ANOVA wherein water type (site), type of anion resin column, and type of cation resin column were whole-plot factors and charcoal was modeled as a subplot factor. For response variables, results were presented graphically with interaction plots (Neter et al. 1990, pp. 681–688, 812–816). All underlying assumptions were verified using standard residual analyses (Neter et al. 1990, pp. 607–614).

Prior field and laboratory experience suggested that charcoal treatments would not markedly affect $\delta^{18}O$ or $\%CO_2$ recovery. Power analysis was used to assess whether effects were of practical significance (Cohen 1990; Muller and Benignus 1992). We believed that in order to justify the expense and difficulty of routine laboratory application of charcoal treatments, at least one or both of two effects of charcoal would have to be demonstrated: (*i*) at least a 15% increase in the CO_2 recovered and (or) (*ii*) at least a 15% increase in the $\delta^{18}O$ value, both relative to untreated controls. Thus, for charcoal effects, we computed retrospectively the power of the tests to detect a 15% increase over controls, with a type I error rate of 0.05. We also computed the smallest detectable effect size for each test. When power was ≥0.90 and the smallest detectable effect size was <15%, we judged statistical power to be sufficient.

Instrumentation

Isotope analysis was performed on Finnigan Mat 251 stable isotope mass spectrometer. Nitrogen isotope values ($\delta^{15}N$) are reported in per mil (‰) relative to atmospheric AIR, which is defined as 0‰; oxygen isotope values ($\delta^{18}O$) are reported relative to the standard VSNOW, also defined as 0‰. The precision for analysis of laboratory standards for $\delta^{15}N$ of KNO_3 was ±0.05‰ (SD) (n = 10) and for $\delta^{18}O$ of $AgNO_3$ was ±0.2‰ (SD) (n = 19). DOC was measured on a total carbon analyzer (Dohrmann) by persulfate and ultraviolet oxidation, with an average analytical coefficient of variation of 15%. The detection limit was 0.2 μmol.

Results

Anion and cation experiment

Comparisons of NO_3^- concentrations before and after passage through the anion column revealed that NO_3^- was quantitatively retained in all cases. Effects of the different experimental variables were strongly dependent on each other as revealed by three-way and two-way interactions (Tables 2 and 3).

Use of a cation column released more DOC, an absolute increment of ~30% for the Ward Creek and Hidden Villa samples, but had little effect on the Sacramento samples (P = 0.0339) (Table 2; Fig. 2). With regard to anion resin type, use of AG2X consistently passed about twice as much DOC through compared with AG1X (P < 0.0001) (Table 2).

The mean $\%NO_3^-$ recovered ranged from 48 to 95%. There were marked effects by both cation and anion treatments (P < 0.0001) (Table 2), and these effects varied by site ($P \leq 0.0001$) (Table 2). This strong three-way interaction is depicted in Fig. 3, in order of increasing effect, ranging from nil at Sacramento to moderate at Hidden Villa to extreme at Ward Creek. For the Sacramento samples, use of a cation column resulted in very small reductions in $\%NO_3^-$, regardless of anion resin type. This contrasted with the Hidden Villa samples; application of the cation column substantially reduced $\%NO_3^-$ in the AG1X treatments but produced no change in the AG2X treatments. At Ward Creek, use of the cation column produced strong and *opposite* effects, depending on the type of anion resin used. The $\%NO_3^-$ showed an absolute increase of 30% when AG2X resin was used with a cation column but declined by 40% when AG1X resin was used.

Mean $\delta^{15}N$ values ranged from +3.30 to +3.85‰ (Fig. 4). The effects of cation and anion treatments varied with site (P = 0.002) (Table 2) and are depicted in Fig. 4 in order of increasing strength among the sites. (The $\delta^{15}N$ would be +3.49‰ if samples contained no NO_3^- of their own.) The $\delta^{15}N$ values for the Hidden Villa samples were similar be-

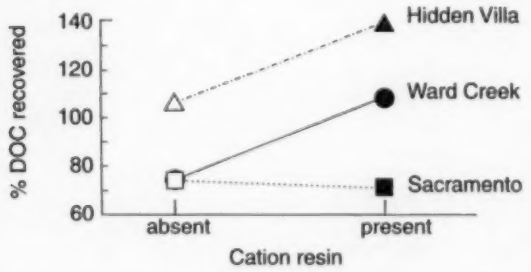
© 1999 NRC Canada

Table 3. Results of ANOVA for experiments on effects of anion resin type (AG1X versus AG2X), cation resin (present or absent), site (Hidden Villa, Ward Creek, and Sacramento), and charcoal (present or absent) on $\%CO_2$ recovery and $\delta^{18}O$.

Experimental unit	Source	df	*P*	
			$\%CO_2$	$\delta^{18}O$
Whole plot	Site	2,24	0.21	0.0003
	Anion	1,24	0.02	0.84
	Site × anion	2,24	0.0005	0.44
	Cation	1,24	0.0005	0.13
	Site × cation	2,24	0.07	0.05
	Anion × cation	1,24	0.04	0.57
	Site × anion × cation	2,24	0.0005	0.23
	Error	24	3960	1082
Split plot	Charcoal	1,23	0.11	0.12
	Site × charcoal	2,23	0.88	0.08
	Anion × charcoal	1,23	0.36	0.69
	Site × anion × charcoal	2,23	0.91	0.87
	Cation × charcoal	1,23	0.98	0.56
	Site × cation × charcoal	2,23	0.39	0.52
	Anion × cation × charcoal	1,23	0.30	0.87
	Site × anion × cation × charcoal	2,23	0.28	0.74
	Error	24	269.6	258.5

Note: For error terms the tabled value is the sum of squares and not a *P* value.

Fig. 2. Effects of site and use of cation column on %DOC recovery. Solid symbols depict the presence of cation resin, and open symbols depict the absence of cation resin. The results of tests for main and interaction effects (site × cation) are given in Table 3.



tween treatments; slightly heavier values were observed with AG2X resin, and the cation column had no effect. For the Ward Creek samples, the opposite response was observed; AG2X samples were lighter, and application of the cation column produced even lighter $\delta^{15}N$ values. Finally, for the Sacramento samples, heavier $\delta^{15}N$ values were obtained with the AG2X treatment.

The $\%CO_2$ recovery ranged from 68 to 120% and was affected by cation and anion treatments, and these effects also showed site differences ($P = 0.0005$) (Table 3). When cation columns were used with AG2X resin, there was a substantial reduction in $\%CO_2$ recovered for the Hidden Villa and Ward Creek samples (Fig. 5). However, the cat-AG2X treatment yielded an increase in $\%CO_2$ for the Sacramento samples.

Differences in $\delta^{18}O$ were due to site effects (Tables 3 and 4); mean $\delta^{18}O$ at Sacramento, +31.34 ± 1.14‰ (SE), was significantly greater than for either Ward Creek (+27.66 ± 0.40‰) or Hidden Villa (+27.04 ± 0.20‰). (Note that if native NO_3^- was not present in the samples, the expected $\delta^{18}O$ would be +26.30‰.) The small magnitude of the site × cation effect ($P = 0.0482$) was due to one high $\delta^{18}O$ value for each of the two cation treatments for the Sacramento samples (+47.97 and +44.90‰). We regard these as outliers, possibly due to a problem when the samples were extracted on the vacuum line.

Use of charcoal did not significantly affect $\%CO_2$ or $\delta^{18}O$, regardless of the treatment combination (Table 3). Mean $\%CO_2$ in charcoal-treated and no-charcoal samples was 86.1 and 83.4%, respectively. Mean $\delta^{18}O$ in charcoal-treated and no-charcoal samples was +29.30 and +28.10‰. We established a priori that differences between charcoal and no-charcoal treatments for $\%CO_2$ and $\delta^{18}O$ must be ≥15% to be of practical importance. Since differences of only 5.3 and 1.9% were observed for $\%CO_2$ and $\delta^{18}O$, respectively, and the power to detect these differences was >0.99, we conclude that charcoal treatment does not affect $\%CO_2$ yield or $\delta^{18}O$.

Additional experiments with the cat-AG2X method

The $\%NO_3^-$ and $\%CO_2$ for the deionized water solution containing the KNO_3 reagent were 79 and 76%, respectively, for the entire cat-AG2X procedure (Table 5). Yields showed an absolute increase of 10% when the $BaCl_2$ step was omitted. In all cases, there was no isotope fractionation; $\delta^{15}N$ = +3.56 ± 0.01‰ (SE) and $\delta^{18}O$ = +24.31 ± 0.03‰ (SE).

The $\%NO_3^-$ for the simulated field samples with the higher load of DOC was 83 ± 0.25%, and the $\%CO_2$ recovery was 73 ± 0.35%. Mean $\delta^{15}N$ of the simulated sample was +5.72 ± 0.07‰ and mean $\delta^{18}O$ was +29.15 ± 0.36‰. The expected $\delta^{15}N$ and $\delta^{18}O$ values of the simulated field sample would be +3.49 and +26.3‰, respectively, if NO_3^- was not associated with the DOC.

© 1999 NRC Canada

Fig. 3. Effects of anion column type and use of cation column on $\%NO_3^-$ at each site. Solid circles depict the presence of cation resin, and open circles depict the absence of cation resin. The results of tests for main and interaction effects (anion × cation × site) are given in Table 3.

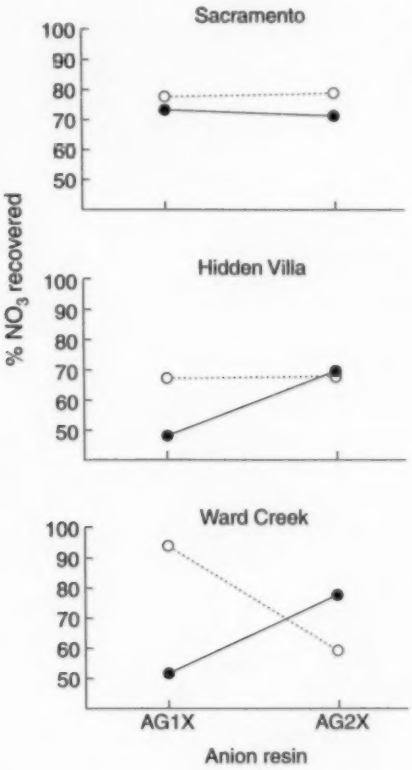
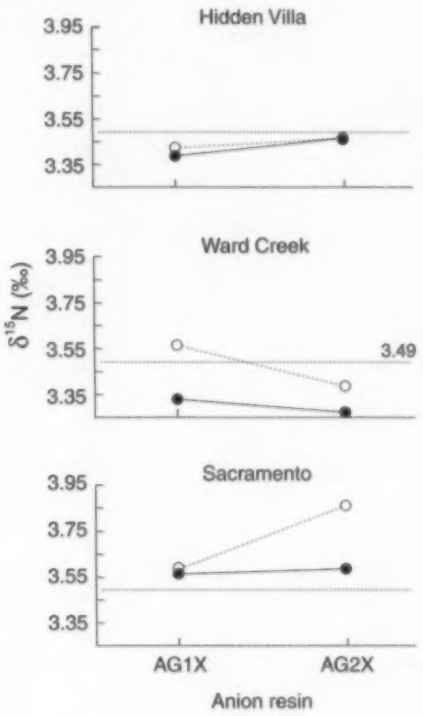


Fig. 4. Effects of anion column type and use of cation column on $\delta^{15}N$ at each site. Solid circles depict the presence of cation resin, and open circles depict the absence of cation resin. The results of tests for main and interaction effects (anion × cation × site) are given in Table 3.



The DOC concentration of the three procedural blanks increased after neutralization with Ag_2O and then declined prior to the freeze-drying step (Table 5). Calculations based on DOC measurements indicated that 4 µmol of carbon was generated from a blank for the entire procedure, and 2 µmol was removed when charcoal was added. A similar pattern was observed for the Sacramento samples. The difference between charcoal and no-charcoal samples was 3.2 µmol of carbon.

Discussion

Three patterns are easily recognized from the experiment: (*i*) differences in $\delta^{18}O$ were due to site effects only, i.e., water chemistry, and not to the anion resin or cation resin columns, (*ii*) twice as much DOC was retained by the AG1X column than by the AG2X column, and (*iii*) the $\%CO_2$ recovery and $\delta^{18}O$ were not affected by charcoal treatment. Fifteen percent was selected as the minimally important difference between charcoal-treated and no-charcoal samples with respect to $\%CO_2$ recovery and $\delta^{18}O$ because this is slightly larger than the typical variation that we have observed for samples processed from snow and snowmelt samples. As differences between charcoal and no-charcoal treatments were all <15%, we conclude that there is no difference between samples with or without charcoal.

To estimate whether the 4 µmol of background carbon introduced by the resins, columns, and reagents may lead to marked fractionation, calculations based on the following assumptions were made: (*i*) oxygen comprises 40% of the average DOC molecule (Thurman 1985) and (*ii*) the $\delta^{18}O$ of the background DOC is +26‰.

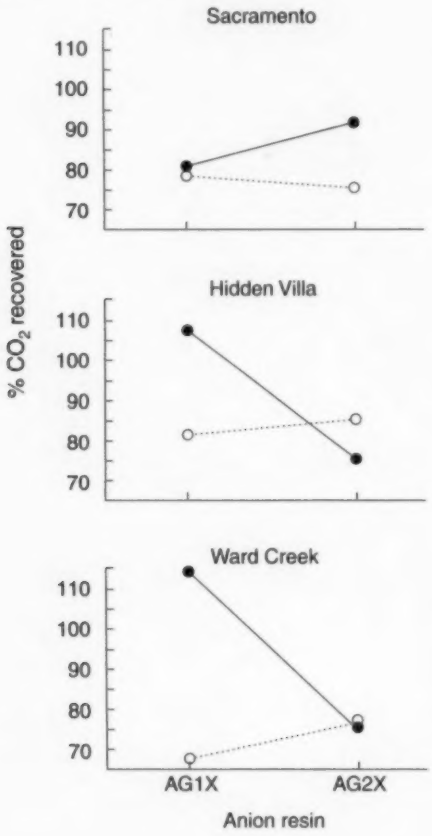
Consider first a sample $\delta^{18}O$ of +40‰ (typical for atmospheric NO_3^-). The isotopic shift for a sample with 50 µmol of NO_3^- will be <0.30‰ (with or without charcoal) and for a 10-µmol sample will be +39.27 and +38.74‰ with and without charcoal, respectively. On the other hand, if the $\delta^{18}O$ is –5‰, the 10-µmol sample will shift to –3.44 and –2.0‰, respectively.

These small shifts would not impair our ability to differentiate hypothetical atmospheric samples from microbial NO_3^- samples, which typically have $\delta^{18}O$ values of –9 through +1‰. Obviously, small samples (≤ 50 µmol of NO_3^-) with negative $\delta^{18}O$ values are more adversely affected by background DOC.

Because of the multiple steps involved and incomplete sample transfer during filtering or freeze-drying, the $\%NO_3^-$ and $\%CO_2$ recoveries are never 100%. This type of loss does not result in isotope fractionation and is very different from having low yields due to incomplete elution of NO_3^- from the anion column. The latter produces high values because

© 1999 NRC Canada

Fig. 5. Effects of anion column type and use of cation column on $\%CO_2$ recovery at each site. Solid circles depict the presence of cation resin, and open circles depict the absence of cation resin. The results of tests for main and interaction effects (anion × cation × site) are given in Table 5.



isotopically enriched NO_3^- comes off the column first (Silva et al. 2000).

This point was illustrated by the observation that the isotope values of the KNO_3 reagent dissolved in deionized water showed no fractionation with $\%NO_3^-$ and $\%CO_2$ of 79 and 89%, respectively. When the $BaCl_2$ was omitted, yields showed an absolute increase of 10% (Table 5). Yields significantly lower than this should be viewed as problematic and may be indicative of fractionation. This criterion was used to evaluate the $\%NO_3^-$ recovery results. Given that the $\%NO_3^-$ recovery for Hidden Villa and Ward Creek was about 50% using the cat-AG1X treatment (significantly lower than for the other three treatments), we recommend against the use of cat-AG1X treatment.

When oxygen other than that from NO_3^- is transferred to the sample, the $\%CO_2$ recovery can exceed 100%. Yields ≥100% were observed for the Hidden Villa and Ward Creek samples with cat-AG1X. Since these recoveries were much higher than with the other three treatments and exceeded the recoveries observed for the sample comprising KNO_3 reagent dissolved in water that was processed through cat-AG2X (Table 5), we must again recommend against the cat-AG1X treatment combination. We also recommend against the AG1X treatment as somewhat lower $\%CO_2$ was observed for Hidden Villa and Ward Creek samples. This leaves us to choose between the AG2X and cat-AG2X treatments.

The $\delta^{15}N$ did not provide clear evidence as to which treatment was optimum because of the site-specific differences in the anion and cation effects (Fig. 3). However, we based our decision on whether to use a cation column with the AG2X resin by examining the %DOC recovery data. Cation columns promoted passage of DOC for the Hidden Villa and Ward Creek samples, i.e., %DOC showed an absolute increase of 30% (Fig. 2). This is consistent with our hope that cation resin would promote DOC passage through the anion column by protonating the DOC and making it less likely to stick to the anion column. The %DOC for the Hidden Villa samples was ≥100%, possibly due to the release of DOC by the columns themselves. Without cation columns, more DOC was retained at these two sites, while %DOC was *slightly* depressed with cation columns for the Sacramento samples. Although our experiments indicated that very little DOC remained in the sample after processing, it is difficult to predict the behavior of every type of DOC that will be encountered in field samples. Therefore, because cation column application enhanced the passage of DOC through the anion column in two out of three cases, and passage only slightly depressed in the third, we recommend use of both cation and AG2X resin.

Larger samples (10–20 L) will expose the anion column to greater amounts of DOC. Because DOC adsorption will inhibit NO_3^- adsorption by anion columns, and may lead to the transfer of DOC oxygen into the sample, it is paramount to prevent DOC accumulation. The wide range of $\%CO_2$ (30–300%) from earlier attempts to collect large-volume samples, using 2-mL columns filled with AG1X (Silva et al. 1999), may have been due to DOC accumulation on the anion columns. That DOC is more tenaciously retained by AG1X resin is not surprising because the relative selectivity of AG1X for anions is about 10 times greater than that of AG2X resin. Our data suggest that this holds true for DOC as well and are consistent with our finding that AG2X resin promoted DOC flow through in all cases.

The success of this method requires careful sample handling because of low NO_3^- concentrations, the numerous laboratory steps, and the small volumes of sample being transferred. Precautions must be taken not to exceed the exchange capacity of the column (6 mequiv.) because this can lead to isotope fractionation. Overloading the column will cause the heavier NO_3^- to exit, resulting in an isotopically "light" sample (Silva et al. 2000). Samples with low NO_3^- concentrations and high Cl^- and SO_4^{2-} concentrations also will be problematic because of the competition for anion sites. Therefore, it is imperative to know the concentrations of all anions and DOC in order to determine the required sample volume and whether the NO_3^- will bleed through the column, i.e., break through. It is also prudent to analyze the eluent after it has passed through the anion column to verify that NO_3^- was efficiently retained.

The cat-AG2X method was used successfully in the Loch Vale, Colorado, watershed for snow, rain, snowmelt, and

© 1999 NRC Canada

Table 4. Mean $\delta^{18}O$ values ± SE (‰) for three sites for each treatment combination.

	Ward Creek		Hidden Villa		Sacramento	
Treatment	Charcoal	No charcoal	Charcoal	No charcoal	Charcoal	No charcoal
AG2X	+28.06±1.23	+26.78±0.51	+27.54±0.58	+27.44±0.43	+29.32±1.51	+28.24±2.69
AG1X	+28.79±1.61	+30.26±1.96	+26.60±0.31	+26.63±0.31	+32.16±2.09	+28.84±0.69
cat-AG2X	+27.24±0.32	+27.02±0.38	+26.82±0.41	+26.72±0.17	+38.39±4.89	+31.96±1.67
cat-AG1X	+26.51±0.61	+26.65±0.32	+26.84±1.18	+27.78±0.70	+33.48±5.70	+28.36±1.60

Table 5. Yields ($\%NO_3^-$ and $\%CO_2$) and mean isotope values ± SE ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- for KNO_3 solutions processed with and without $BaCl_2$ using the cat-AG2X method and the simulated field sample containing KNO_3 and 2400 µmol of DOC.

Parameter	$\%NO_3^-$	$\%CO_2$	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
With $BaCl_2$	79 (n = 10)	76 (n = 6)	+3.53±0.04	+25.92±0.26
Without $BaCl_2$	89 (n = 12)	86 (n = 6)	+3.49±0.04	+26.01±0.18
Simulated field sample	83 (n = 12)	73 (n = 12)	+5.72±0.07	+29.15±0.36

Table 6. Water chemistry, $\%NO_3^-$ and $\%CO_2$ recovery, and $\delta^{15}N$ and $\delta^{18}O$ values for alpine samples collected in Loch Vale, Colorado.

Sample type	Volume (L)	NO_3^- (µM)	NO_3^- loaded (µmol)	$\%NO_3^-$	$\%CO_2$	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	DOC (µM)	SO_4^{2-} (µM)	Cl^- (µM)
Stream	6	28	168	74	96	+1.8	+7.3	104	85	3.6
Rain	14.5	8.4	122	77	85	+0.6	+39.8	160	10.8	1.6
Snow	14	8.9	125	80	75	+0.8	+60.2	178	7.8	1.6
Snow	20.5	8.9	183	93	65	+2.2	+64.4	205	25.1	1.3
Snow	10	8.7	87	78	83	+1.0	+59.3	271	17.75	3.2
Snow	19	7.4	141	75	94	–0.2	+61.6	na	7.9	1.2
Snow	17.6	10.3	181	100	67	+4.3	+61.2	na	10.9	1.3
Snow	16.6	6.7	111	84	81	–1.8	+62.0	na	6.5	2.1
Snow	17.3	16.3	282	84	75	+6.0	+66.1	374	12.4	1.6

Note: 1 ppm DOC ≈ 80 µM DOC (Qian and Mopper 1996). na, not available.

spring samples. Examples of recovery ($\%NO_3^-$ and $\%CO_2$) and stable isotope values for these types of samples are shown in Table 6. This method has subsequently been applied to similar samples in the Catskills, New York, Sleepers River, Vermont, Hubbard Brook, New Hampshire, and Sierra Nevada, California (Kendall 1998).

Conclusions

The cat-AG2X method facilitates collection of water samples with low NO_3^- concentrations (1–10 µM) for stable isotope analysis by accommodating large sample volumes (20–70 L). Key to the success of this method is to offset DOC accumulation on the anion column. This was accomplished by putting a cation column in front of the anion column. It is critical to prevent accumulation because DOC can clog the anion column, occupying sites that would otherwise be available for NO_3^-, and because oxygen from DOC may inadvertently be transferred to the CO_2 sample. Experiments to determine whether sample DOC was transferred into the CO_2 portion of the sample and whether charcoal should be used to remove it revealed that charcoal had no clear effect on yields of CO_2 or $\delta^{18}O$. Given that charcoal removed only 2 µmol of DOC, there is no clear justification for using charcoal. Moreover, given that charcoal adsorbs NO_3^-, it should especially be avoided with small samples.

The use of a filter cartridge and larger mesh size anion resin, increasing the resin volume to 5 mL, using AG2X anion resin (100–200 mesh), and application of the cation column greatly reduced the time needed to collect field samples. Sample recovery after processing has also been more consistent. Because samples can be collected in the field and the need to transport water samples is eliminated, it is now possible to obtain samples from access-limited sites such as alpine watersheds, giving researchers the opportunity to identify NO_3^- sources and transformations in snow, rain, snowmelt, and streams.

Acknowledgments

We thank Robert Drimmie, Eric Caldwell, James Sickman, and Len Wassenaar for their reviews.

References

Amberger, A., and Schmidt, H.L. 1987. Naturliche Isotopengehalte von Nitrat als Indikatoren für dessen Herkunft. Geochem. Cosmochim. Acta, **51**: 2699–2705.

Aravena, R., and Robertson, W.D. 1998. Use of multiple isotope tracers to evaluate denitrification in groundwater: case study of nitrate from a large-flux septic system plume. Ground Water, **36**: 975–982.

© 1999 NRC Canada

Aravena, R., Evans, M.L., and Cherry, J.A. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Ground Water, **31**: 180–186.

Böhlke, H.L., Ericksen, G.E., and Revesz, K. 1997. Stable isotope evidence for an atmospheric origin of desert nitrate deposits in northern Chile and southern California, U.S.A. Chem. Geol. Isot. Geos. **136**: 135–152.

Böttcher, J., Strebel, O., Voerkelius, S., and Schmidt, H.L. 1990. Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. J. Hydrol. **114**: 413–424.

Cohen, J. 1990. Things I have learned (so far). Am. Psychol. **45**: 1304–1312.

Durka, W., Schulze, E.D., Gebauer, G., and Voerkelius, S. 1994. Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements. Nature (Lond.), **372**: 765–767.

Harrington, R.R., Kennedy, B.P., Chamberlain, C.P., Blum, J.D., and Folt, C.L. 1998. ^{15}N enrichment in agricultural catchments: field patterns and applications to tracking Atlantic salmon (*Salmo salar*). Chem. Geol. **147**: 281–294.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. *In* Isotope-tracers in catchment hydrology. *Edited by* C. Kendall and J.J. McDonnel. Elsevier, Amsterdam, The Netherlands. pp. 534–569.

Kendall, C., and Grim, E. 1990. Combustion tube method for measurements of nitrogen isotope ratios using calcium oxide for total removal of carbon dioxide and water. Anal. Chem. **62**: 526–529.

Kendall, C., Campbell, D.H., Burns, D.A., Shanley, J.B., Silva, S.R., and Chang, C.C.Y. 1995. Tracing sources of nitrate in snowmelt runoff using the oxygen and nitrogen isotopic compositions of nitrate. *In* Biochemistry of seasonally snow-covered catchments. Proceedings of a symposium held during the XXI Assembly of the International Union of Geodesy and Geophysics at Boulder, Co. July 1995. *Edited by* K.A. Tonnessen, M.W. Williams, and M. Tranter. Publ. No. 228. IAHS Press, Insitute of Hydrology, Wallingford, Oxfordshire, U.K. pp. 339–347.

Muller, K.E., and Benignus, V.A. 1992. Increasing scientific power with statistical power. Neurotoxicol. Teratol. **14**: 211–219.

Neter, J., Wasserman, W., and Kutner, M.H. 1990. Applied statistical linear models. Richard D. Irwin, New York.

Qian, J., and Mopper, K. 1996. Automated high-performance, high-temperature combustion total organic carbon analyzer. Anal. Chem. **68**: 3090–3097.

Revesz, K., Böhlke, J.K., and Yoshinari, T. 1997. Determination of $\delta^{18}O$ and $\delta^{15}N$ in nitrate. Anal. Chem. **69**: 4375–4380.

Silva, S.R., Kendall, C., Wilkison, D.H., Ziegler, A.C., Chang, C.C.Y., and Avanzino, R.J. 2000. A new method for collection of nitrate from fresh water and analyis of the nitrogen and oxygen isotope ratios. J. Hydrol. In press.

Thurman, E.M. 1985. Organic geochemistry of natural waters. Martinus Nijoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands.

Voerkelius, S. 1990. Isotopen diskrimminierungen bie der Nitrifikation und denitrifikation: Grundlagen and Andwendungen der Herkunfts-Zuordungen von Nitrat und Distick-stoffmonoxid. Ph.D. dissertation. Technical University of Munich, Munich, Germany.

Wassenaar, L.I. 1995. Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. Appl. Geochem. **10**: 391–405.

© 1999 NRC Canada

Planktivory by the predacious cladoceran *Bythotrephes longimanus*: effects on zooplankton size structure and abundance

Eva Wahlström and Erika Westman

Abstract: In order to study density-dependent effects of invertebrate planktivory, four different densities of *Bythotrephes longimanus* were inoculated into mesocosm enclosures with a mixed zooplankton community. Changes in size structure and abundance of zooplankton and phytoplankton communities were recorded over a period of 3 weeks. High densities of *Bythotrephes* were able to reduce total zooplankton abundance, which was mainly due to a decrease in the density of the relatively large species *Holopedium gibberum*. The density of the smaller species *Bosmina longirostris* was also reduced with increasing densities of *Bythotrephes*, whereas rotifer abundance remained largely unaffected. The mean size of *Holopedium* increased with increasing densities of *Bythotrephes*. Despite the decrease in total zooplankton biomass in high-*Bythotrephes* treatments, no effect of *Bythotrephes* density on primary producers was observed. Our experiment shows that predacious cladocerans may reduce macrozooplankton biomass, large as well as small species. Predation from invertebrate planktivores results in a zooplankton community consisting of larger individuals. Comparing our experimental densities with densities of *Bythotrephes* found in natural systems suggests that invertebrate planktivores may influence size structure and abundance of zooplankton communities even in lakes with planktivorous fish.

Résumé : Pour étudier les effets de diverses densités d'invertébrés planctivores, on a introduit quatre densités différentes de *Bythotrephes longimanus* dans des mésocosmes en enclos renfermant une communauté mixte de zooplancton. On a mesuré sur trois semaines les changements dans l'abondance et la composition par taille des communautés de zooplancton et de phytoplancton. Les fortes densités de *Bythotrephes* avaient pour effet de réduire l'abondance totale de zooplancton, réduction liée principalement à une diminution de la densité de *Holopedium gibberum*, une espèce de taille relativement grande. La densité de *Bosmina longirostris*, une espèce plus petite, variait également en raison inverse des densités de *Bythotrephes*, tandis que l'abondance des rotifères variait très peu. La taille moyenne de *Holopedium* variait en raison directe des densités de *Bythotrephes*. Malgré la diminution de la biomasse totale de zooplancton quand les densités de *Bythotrephes* étaient élevées, on a observé que la densité de cette espèce n'avait pas d'effet sur les producteurs primaires. Notre expérience montre que les cladocères prédateurs peuvent réduire la biomasse des espèces tant grandes que petites de macrozooplancton. La prédation par les invertébrés planctivores a pour effet d'accroître la taille des zooplanctontes dans le milieu. La comparaison de nos densités expérimentales avec les densités de *Bythotrephes* observées dans les milieux naturels laissent penser que les invertébrés planctivores peuvent influer sur les abondances et la composition par tailles des communautés zooplanctoniques, même dans les lacs renfermant des poissons planctivores.

[Traduit par la Rédaction]

Introduction

It is well established that vertebrate planktivores, feeding selectively on large zooplankton, cause zooplankton communities to be dominated by small-sized zooplankton (Hall et al. 1976; Black and Hairston 1988; Lazzaro et al. 1992; Rodríguez et al. 1993). In contrast, invertebrate planktivores forage preferentially on smaller zooplankton and rotifers, and this may result in changes where small species are reduced or eliminated and zooplankton communities become dominated by large species (Neill 1988*a*; Vanni 1988; Hanazato and Yasuno 1989; Yan and Pawson 1997). The magnitude of the effect of invertebrate planktivores on zooplankton community structure and density is usually considered small relative to vertebrate planktivores (i.e., fish) (Zaret 1980; Gliwicz and Pijanowska 1989; Hansson and Tranvik 1996). This fact may be due to several factors such as the higher feeding capacity and broader prey size range of fish compared with invertebrate planktivores but also the larger interspecific similarity in prey preference among vertebrate planktivores compared with invertebrate planktivores (Zaret 1980; Gliwicz and Pijanowska 1989).

In natural systems with both planktivorous fish and planktivorous zooplankton, the effect of invertebrate planktivory is reduced, as predacious zooplankton are a selected prey of planktivorous fish (Elser et al. 1987; Black and Hairston 1988; Bur and Klarer 1991). As a result, some studies suggest that the effects of invertebrate planktivory on

Received January 15, 1999. Accepted June 22, 1999.
J14977

E. Wahlström[1] and E. Westman. Department of Ecology and Environmental Sciences, Umeå University, S-901 87 Umeå, Sweden.

[1]Author to whom all correspondence should be addressed.
e-mail: eva.wahlstrom@animecol.umu.se

© 1999 NRC Canada

zooplankton community structure and abundance will only be important in systems where fish planktivory is low or absent (Black and Hairston 1988; Lehman 1991). However, several observational studies of systems with both fish and invertebrate planktivores show invertebrate planktivores exerting a significant effect on zooplankton community structure (Makarewicz et al. 1995; Yan and Pawson 1997).

One reason for this inconsistency is that there are few experimental studies on invertebrate planktivory. Experiments on invertebrate predators have been reported but are largely restricted to studies on Chaoborus larvae (Neill 1988*a*; Riessen et al. 1988; Vanni 1988; Hanazato and Yasuno 1989; MacKay et al. 1990). In contrast, experimental studies covering the cladoceran predators, such as *Bythotrephes* and *Leptodora*, are lacking and evidence for an impact of these invertebrate predators on zooplankton community is largely indirect and, at best, based on bioenergetic calculations (Berg and Garton 1988; Lunte and Luecke 1990; Lehman 1991; Lehman and Cáceres 1993; Burkhardt and Lehman 1994; Makarewicz et al. 1995; Yurista and Schulz 1995; Yan and Pawson 1997).

To experimentally test for the potential effects of the predacious cladoceran *Bythotrephes longimanus* on zooplankton communities, we performed an experimental study in mesocosms. Specifically, we tested for (*i*) the density dependence of the interaction between *Bythotrephes* and the rest of the zooplankton community and (*ii*) the effect of *Bythotrephes* density on zooplankton size structure. In addition, we also studied the effect of different densities of *Bythotrephes* on primary producers. We predicted a reduction in density of smaller zooplankton species and an increased mean size of zooplankton with increasing densities of *Bythotrephes*. Effects on primary producers were predicted to be minor because large, and more efficient, grazing zooplankton should be invulnerable to predation by this invertebrate planktivore. Finally, the densities of *Bythotrephes* used in the experiment were compared with densities found in the literature to evaluate the relevance of the effects of *Bythotrephes* seen in the mesocosm experiment to natural systems with fish.

Material and methods

Site description

The experiment was conducted in 16 transparent plastic enclosures in 1997 in Lake Abborrtjärn 3, belonging to an experimental lake area in central Sweden (for a further description, see Persson et al. 1996). The enclosures were 8 m deep, had a diameter of 1.6 m, and extended 30 cm above the water level (volume 16 m^3). Each plastic bag was connected to a wooden frame with styrofoam as a floating device. The frames were placed in rows of four, and within each row, frames were spaced out at a distance of 0.5 m from each other in an east–west position. Each enclosure was covered with a nylon net with a mesh size of 0.5 mm to prevent adult *Chaoborus* from laying eggs in the enclosures. On May 29–31, the enclosures were filled with lake water filtered through a 50-μm-mesh nylon net to avoid macrozooplankton but allow phytoplankton into the enclosures.

Inoculation

One week after filling (June 9–12), the enclosures were inoculated with zooplankton. Zooplankton were collected from four different adjacent lakes (including the lake where the experiment was conducted) with an 80-μm-mesh zooplankton net (diameter 0.5 m). The zooplankton from each lake were brought together in a large barrel and examined to avoid inoculation of any of the present invertebrate planktivores, *Bythotrephes longimanus*, *Leptodora kindtii*, and *Chaoborus* spp. Before inoculation of zooplankton in the enclosures, the zooplankton were gently mixed and each enclosure was inoculated with the same volume of the zooplankton mix, resulting in densities in the enclosures similar to average densities in the four lakes. One week later (June 17), the 16 enclosures were randomly assigned to one out of four treatments within each row. The treatments were addition of *Bythotrephes longimanus* at 0, 100, 600, and 1000 individuals per enclosure, which correspond to 0, 6.25, 37.5, and 62.5 individuals·m^{-3} (hereafter 0B, 6B, 40B, and 60B). The densities used in the experiment corresponded to average natural densities in Lake Abborrtjärn 2 (a closely situated lake in the experimental lake area) (6B) at this time of the season, average densities from a former enclosure experiment with a *Bythotrephes* treatment (40B, E. Wahlström, unpublished manuscript), and densities found in other systems based on the literature (60B, Berg and Garton 1988).

Sampling

The first samples of zooplankton and phytoplankton were taken 1 day prior to the *Bythotrephes* inoculation. Thereafter, zooplankton were sampled twice a week and phytoplankton once a week for a period of 3 weeks, starting 1 week after the inoculation with *Bythotrephes*. Macrozooplankton were sampled with vertical net hauls using a 100-μm-mesh nylon net (diameter 25 cm). The position of the thermocline was determined with a thermistor at each sampling occation and the samples were divided into epilimnetic and hypolimnetic samples. Subsequent analysis demonstrated that separation of zooplankton samples into epilimnetic and hypolimnetic samples did not add any information (epilimnetic and hypolimnetic samples were qualitatively similar, although biomass in the hypolimnion was lower); therefore, all statistical analyses of macrozooplankton were performed on the average total biomass in each enclosure. Microzooplankton (rotifers, copepod nauplii) were sampled using a Rhuttner water sampler; 1.5 L of water was taken at each 1 m from the surface to a depth of 7 m (total volume 12 L). The sampled volume was filtered through a 25-μm-mesh nylon net and transferred to a 100-mL glass bottle. Zooplankton samples were preserved with Lugol's solution. In the laboratory, the macrozooplankton were classified, counted, and the body lengths of 20 individuals (all, if fewer) of each taxon from each sample measured under an inverted microscope. Lengths were then transformed to biomass using length to weight regressions (Bottrell et al. 1976). Microzooplankton (rotifers) were classified and individuals counted.

Bythotrephes were sampled with a 0.5-mm-mesh net (diameter 60 cm) two times during the experiment 2 weeks after the start and at the termination of the experiment. The sampled individuals were preserved for length measurements and determination of developmental stage.

Phytoplankton biomass was estimated as chlorophyll *a* content. Samples for phytoplankton were taken at each 1 m from the surface to a depth of 6 m (including the metalimnion) with a Rhuttner water sampler. The samples were mixed and two volumes of each water sample (1.0–2.0 L), one of which had previously been filtered through a 35-μm-mesh nylon net, were filtered through Whatman GF/C filters. The filters were dried and frozen (–25°C) until further analyses. Algae on the filters was extracted in methanol and the absorbance at 665 and 750 nm was measured in a spectrophotometer.

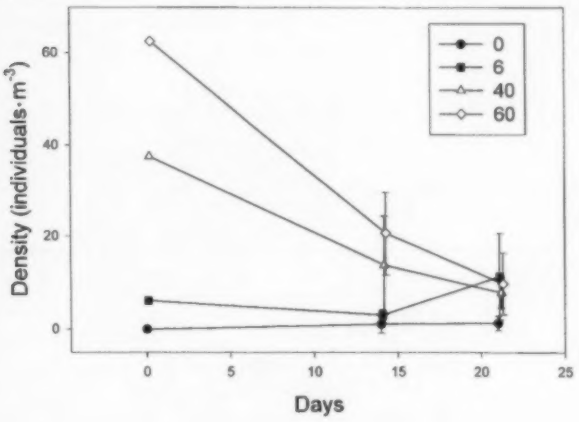
Results

Bythotrephes

The densities of *Bythotrephes* decreased in the 40B and

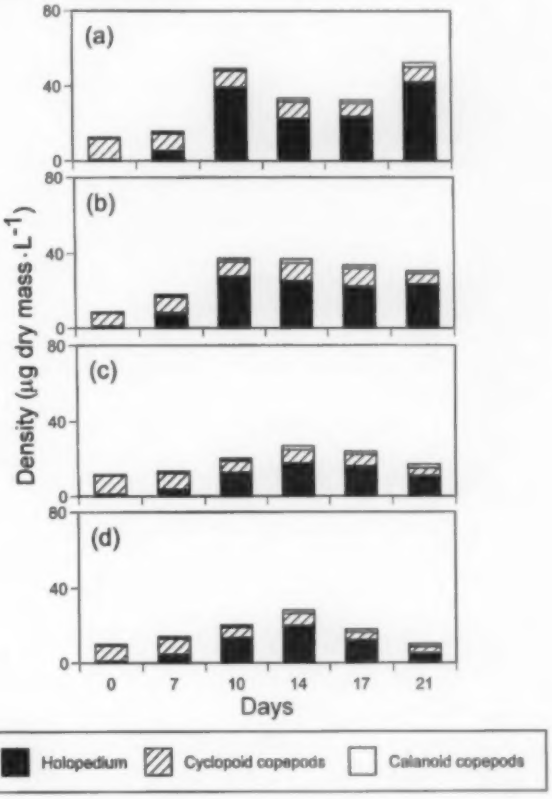
© 1999 NRC Canada

Fig. 1. Changes in densities of *Bythotrephes longimanus* within the course of the experiment. Data are means ± 1 SD of four replicates.



60B treatments during the experiment, whereas a small increase was observed in the 6B treatments (Fig. 1). A slight increase was also observed in the 0B treatment. This may be due to a sampling error caused by the failure to rinse the net thoroughly between enclosures at the first sampling occasion, resulting in unintended inoculations of *Bythotrephes* into two of the 0B replicates. The density of *Bythotrephes* was higher in the 60B treatment than in the 0B and 6B treatments after 2 weeks of the experiment (ANOVA, $F_{3,12}$ = 6.92, P = 0.006; Scheffe post hoc test: 0B–6B, P = 0.98; 0B–40B, P = 0.14; 0B–60B, P = 0.02; 6B–40B, P = 0.25; 6B–60B, P = 0.03; 40B–60B, P = 0.61). No differences in *Bythotrephes* densities between treatments were present at the end of the experiment (ANOVA: $F_{3,12}$ = 2.18, P = 0.143).

Fig. 2. Biomass of *Holopedium gibberum*, cyclopoid copepods, and calanoid copepods versus time for mesocoms with (*a*) 0, (*b*) 6, (*c*) 40, and (*d*) 60 *Bythotrephes longimanus*·m^{-3}. Data are means of four replicates.



Macrozooplankton

The species composition was similar in all enclosures and consisted of *Holopedium gibberum*, cyclopoid and calanoid copepods, and, at very low densities, *Daphnia longispina*, *Bosmina longisrostris*, and *Ceriodaphnia quadringula* (Figs. 2 and 3). The dominant species in all treatments was *Holopedium gibberum*. Zooplankton biomass increased in all enclosures during the first 10–14 days of the experiment, decreasing thereafter until the end of the experiment, except for the 0B treatment where zooplankton biomass increased on the last sampling occasion. Total zooplankton biomass was highest in the 0B treatment, intermediate in the 6B treatment, and lowest in the 40B and 60B treatments (Fig. 2; Table 1). The biomass of *Holopedium* decreased with increasing *Bythotrephes* density (Fig. 2; Table 1). No effect of *Bythotrephes* density on the biomass of cyclopoid copepods or calanoid copepods was observed (Fig. 2; Table 1). The biomass of *Bosmina* decreased with increasing *Bythotrephes* density (Fig. 3; Table 1). *Daphnia* and *Ceriodaphnia* were almost totally eliminated in all treatments after the first week of the experiment (Fig. 3).

The mean size of *Holopedium* increased in all treatments during the experiment, but the increase was higher in the high-*Bythotrephes* treatment than in the low-*Bythotrephes* treatments (Fig. 4*a*) (repeated-measures ANOVA: *Bythotrephes* density, $F_{3,12}$ = 43, P < 0.001; time, $F_{5,60}$ = 45, P < 0.001; *Bythotrephes* density × time, $F_{15,60}$ = 3.0, P = 0.001). A slight increase in the size of cyclopoid copepods was observed in all treatments during the experimental period, but no treatment effect was found (Fig. 4*b*) (repeated-measures ANOVA: *Bythotrephes* density, $F_{3,12}$ = 0.77, P = 0.53; time, $F_{5,60}$ = 8, P < 0.001; *Bythotrephes* density × time, $F_{15,60}$ = 1.7, P = 0.085). A similar pattern was found for the mean size of calanoid copepods (Fig. 4*c*) (repeated-measures ANOVA: *Bythotrephes* density, $F_{3,12}$ = 0.58, P = 0.64; time, $F_{6,60}$ = 14, P < 0.001; *Bythotrephes* density × time, $F_{15,60}$ = 0.64, P = 0.83). The number of measured individuals of the zooplankton species *Bosmina*, *Daphnia*, and *Ceriodaphnia* was too low to allow an analysis of differences in the mean size of these species between treatments.

The abundance of copepod nauplii (both cyclopoid and calanoid) increased in all treatments during the first 2 weeks of the experiment and thereafter remained fairly constant (Fig. 5). No difference in abundance was found between treatments (repeated-measures ANOVA: *Bythotrephes* density, $F_{3,12}$ = 0.13, P = 0.94; time, $F_{5,60}$ = 103, P < 0.001; *Bythotrephes* density × time, $F_{15,60}$ = 0.98, P = 0.49).

Rotifers

The total rotifer abundance increased during the first 7–

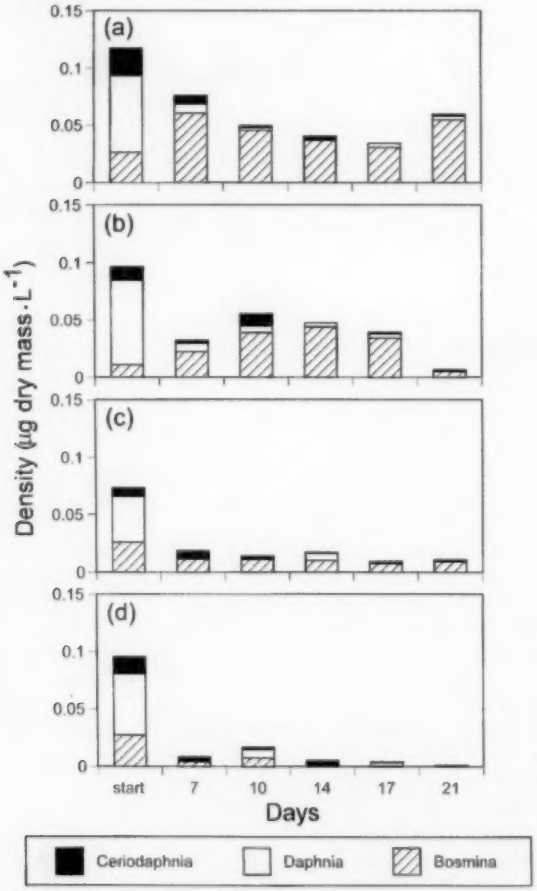
© 1999 NRC Canada

Table 1. Repeated-measures ANOVA of the effects of different *Bythotrephes* densities on total zooplankton biomass and biomass of separate zooplankton taxa.

		Total zooplankton		*Holopedium*		*Bosmina*		Cyclopoida		Calanoida		*Daphnia*		*Ceriodaphnia*	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Between subjects															
Bythotrephes density	3	5.44	0.014	8.67	0.002*	10.2	0.001*	2.72	0.091	0.26	0.851	0.45	0.725	0.48	0.702
Within subjects															
Time	5	15.2	0.000	60.5	0.000	0.27	0.929	2.62	0.033	9.49	0.000	18.7	0.000	6.78	0.000
Bythotrephes × time	15	1.48	0.142	1.78	0.059	0.98	0.491	0.64	0.832	0.35	0.987	0.33	0.990	0.72	0.755

Note: Data were log transformed prior to statistical analyses to stabilize variances.
*P value of between-subject factor significant after adjustment according to a sequential Bonferroni technique.

Fig. 3. Biomass of *Ceriodaphnia quadrangula*, *Daphnia longispina*, and *Bosmina longirostris* versus time for mesocosms with (*a*) 0, (*b*) 6, (*c*) 40, and (*d*) 60 *Bythotrephes longimanus*·m^{-3}. Data are means of four replicates.



10 days of the experiment and thereafter decreased in all enclosures (Fig. 6). No effect of different *Bythotrephes* densities on total rotifer abundance was observed (Table 2). There was no effect of different *Bythotrephes* densities on the abundance of separate rotifer taxa, except for the colonial rotifer *Conochilus* (Fig. 6; Table 2). At the end of the experiment, *Conochilus* was more abundant in the treatments with high *Bythotrephes* densities.

Phytoplankton

Total phytoplankton biomass decreased drastically during the first week of the experiment in all treatments (Fig. 7). There was a tendency (although not significant at the P = 0.05 level) for a continued decrease in the 0B and 6B treatments, whereas the 40B and 60B treatments remained relatively constant until the end of the experiment (Fig. 7) (repeated-measures ANOVA: *Bythotrephes* density, $F_{3,9}$ = 1.0, P = 0.44; time, $F_{3,27}$ = 163, P < 0.001; *Bythotrephes* density × time, $F_{9,27}$ = 2.0, P = 0.08). No difference between treatments was observed for the biomass of algae smaller than 35 µm (repeated-measures ANOVA: *Bythotrephes* density, $F_{3,9}$ = 1.7, P = 0.23; time, $F_{3,27}$ = 86, P < 0.001; *Bythotrephes* density × time, $F_{9,27}$ = 1.5, P = 0.20).

Discussion

High densities of *Bythotrephes* were able to reduce total macrozooplankton biomass. A few studies have been able to show a general reduction in total macrozooplankton abundance by invertebrate predators, and these are studies on *Chaoborus* larvae (Neill 1988*b*; Riessen et al. 1988; Hanazato and Yasuno 1989; MacKay et al. 1990). The dominant zooplankton species in the enclosures was *Holopedium*, and the major effect of *Bythotrephes* was a reduction in *Holopedium* biomass. Furthermore, *Bythotrephes* almost eliminated *Bosmina* in all *Bythotrephes* treatments. Our experiment thus clearly shows that the density of larger zooplankton species as well as smaller species may be reduced by the presence of invertebrate planktivores. This is partly in contradiction to many studies of invertebrate planktivory that show a reduction of small zooplankton species and a corresponding increase of larger species (Hanazato and Yasuno 1989; Lehman 1991; Yan and Pawson 1997). For example, Yan and Pawson (1997) showed a dramatic decline in or disappearance of small species (*Bosmina*, *Chydorus*, *Diaphanosoma*), whereas the abundance of *Holopedium* increased after the invasion of *Bythotrephes cederstroemi*. *Holopedium* has also been shown to be relatively resistant to invertebrate planktivory (Allan 1973; Vinyard and Menger

© 1999 NRC Canada

Fig. 4. Changes in size (mean ± 1 SD) over time for (*a*) *Holopedium*, (*b*) cyclopoid copepods, and (*c*) calanoid copepods in treatments with different densities of *Bythotrephes longimanus*.

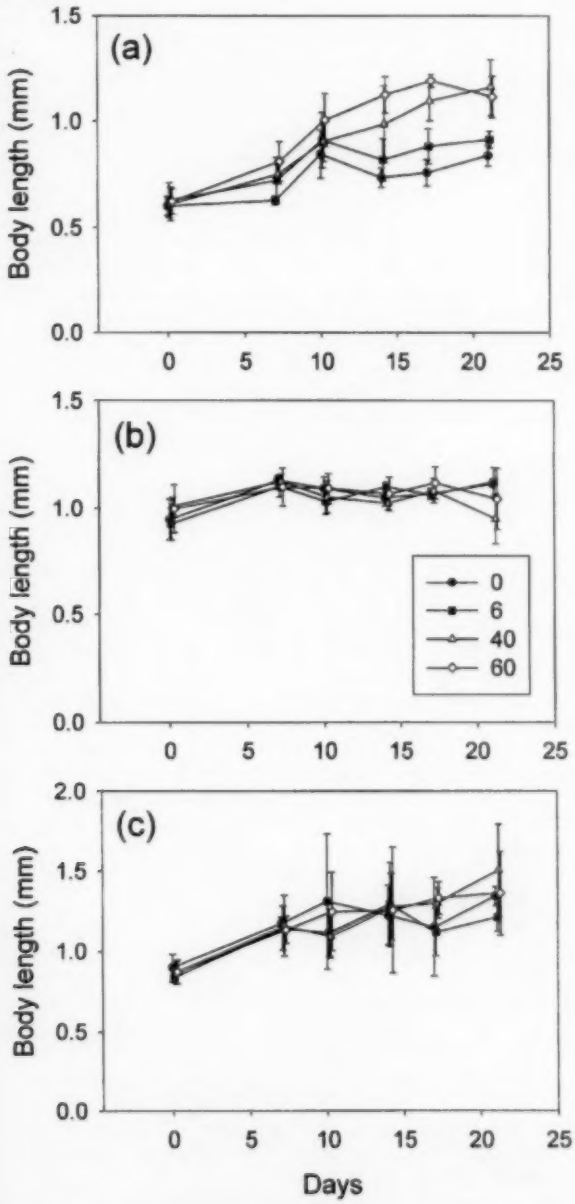


Fig. 5. Changes in density over time for copepod nauplii (both cyclopoid and calanoid) in treatments with different densities of *Bythotrephes longimanus*. Data are means ± 1 SD of four replicates.

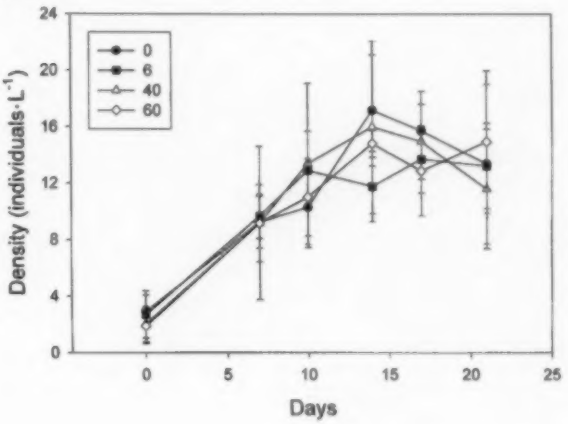
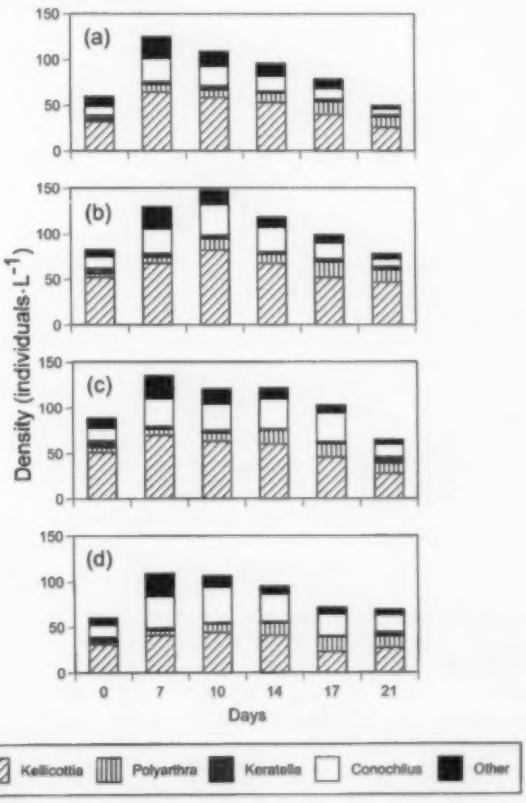


Fig. 6. Density of rotifers versus time for mesocoms with (*a*) 0, (*b*) 6, (*c*) 40, and (*d*) 60 *Bythotrephes longimanus*·m^{-3}. Data are means of four replicates.



1980; Vanni 1988), which might be due to its gelatinous cover and large size. Recently moulted individuals and newly released neonates, however, have only a thin gelatinous cover (Hamilton 1958). It is possible that *Bythotrephes* feeds mainly on these small individuals, thereby affecting the recruitment to large sizes of *Holopedium*. This hypothesis is in agreement with the observation that the mean size of *Holopedium* was larger in treatments with high *Bythotrephes* density compared with treatments with zero or low *Bythotrephes* density. This pattern is also in accordance with most studies of invertebrate planktivory resulting in in-

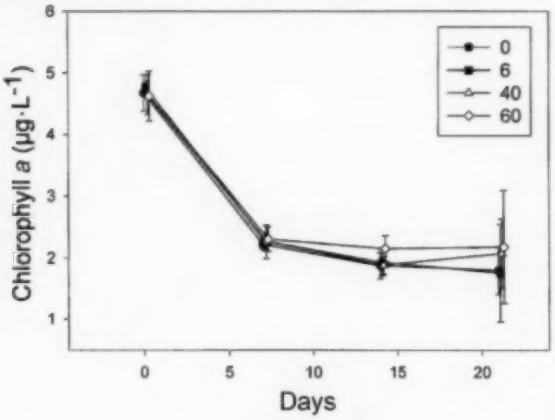
© 1999 NRC Canada

Table 2. Repeated-measures ANOVA of the effects of different *Bythotrephes* densities on total rotifer abundance and abundance of separate rotifer taxa.

		Total rotifers		*Kellikottia*		*Polyarthra*		*Keratella*		*Conochilus*	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Between subjects											
Bythotrephes density	3	1.94	0.176	1.66	0.229	0.17	0.912	0.74	0.548	8.74	0.002*
Within subjects											
Time	5	16.3	0.000	10.0	0.000	37.5	0.000	9.71	0.000	18.0	0.000
Bythotrephes × time	15	0.47	0.949	0.69	0.784	1.20	0.298	1.05	0.421	0.62	0.847

Note: Data was log transformed prior to statistical analyses to stabilize variances.
*P value of between-subject factor significant after adjustment according to a sequential Bonferroni technique.

Fig. 7. Changes in biomass of total phytoplankton, estimated as chlorophyll *a*, over time in treatments with different densities of *Bythotrephes longimanus*. Data are means ± 1 SD of four replicates.



creases in zooplankton mean size (Neill 1988*a*; Vanni 1988; Hanazato and Yasuno 1989; Lehman 1991; Yan and Pawson 1997). An alternative or complementary explanation of size-selective predation by *Bythotrephes* for the observed increase in mean size of *Holopedium* with increasing *Bythotrephes* density could be competitive release and a concomitant increase in individual growth of *Holopedium*.

We did not observe any differences in either total rotifer abundances or copepod biomass between treatments of different *Bythotrephes* densities. Rotifers (*Asplanchna*) have been suggested as a favoured prey of *Bythotrephes cederstroemi* (Mordukhai-Boltovskaia 1958; Vanderploeg et al. 1993). The only rotifer species that was affected by *Bythotrephes* density in our experiment was the colonial *Conochilus*, which increased in abundance with increasing densities of *Bythotrephes*. A positive correlation between *Conochilus* and *Bythotrephes cederstroemi* abundances has previously been observed (Sandgren and Lehman 1990; Branstrator and Lehman 1991). This was suggested to be an effect of decreased densities of *Leptodora* coinciding with increased abundance of *Bythotrephes* rather than an effect of increased density of *Bythotrephes* per se (Sandgren and Lehman 1990; Branstrator and Lehman 1991). In addition, *Conochilus* has a mucus sheet enclosing the colony, which may allow this rotifer to escape predation from *Bythotrephes* (Wallace and Snell 1991). At present, we are unable to explain this result; several direct and indirect effects between *Bythotrephes* and its planktonic prey may be possible, but their investigation is beyond the scope of this study. In contrast with rotifers, copepods have been suggested to be a nonpreferred prey for *Bythotrephes cederstroemi* mainly due to the high escape ability of copepods (Mordukhai-Boltovskaia 1958; Vanderploeg et al. 1993). A study of the physiological economics of *Bythotrephes cederstroemi* further supports the hypothesis that copepods are only a minor component of the predator's diet (Lehman and Branstrator 1995). However, empirical data on diets of *Bythotrephes* are very incomplete. This primarily relates to the difficulty in performing analyses of the stomach contents of *Bythotrephes*, which contain only liquid food (Mordukhai-Boltovskaia 1958; Vanderploeg et al. 1993).

The densities of *Bythotrephes* decreased in the 60B and 40B treatments, whereas a slight increase was observed in the 6B treatment at the end of the experiment, resulting in approximately the same densities of *Bythotrephes* in all treatments at the end of the experimental period. The general convergence towards 6B could be a result of resource reduction due to the observed decrease in *Holopedium* abundance. It could also be argued that the convergence pattern was a result of a juvenile bottleneck where only late-instar stages of *Bythotrephes* forage successfully on larger species, whereas neonates and early instars may depend on smaller prey. Juvenile bottlenecks due to size-specific prey preference have been observed in *Chaoborus* larvae (Neill 1988*a*, 1988*b*). We did in fact observe a reduction in or elimination of small cladocerans in all treatments with *Bythotrephes*, but no effect of *Bythotrephes* density was observed on the abundance of rotifers, which might represent an alternative small prey. Another argument against the bottleneck hypothesis is that the size difference between juvenile and adult *Bythotrephes longimanus* was too small. There was no significant difference in body length between instar I and instar III of *Bythotrephes* when inoculated into the enclosures (E. Westman, personal observation).

There was no significant effect of different *Bythotrephes* densities on primary producers, except for a tendency for a continued decrease in phytoplankton in low-density treatments at the termination of the experiment. In spite of the evident decrease in grazing macrozooplankton due to predation by invertebrate planktivores, few studies of invertebrate planktivores have been able to detect effects on the biomass of primary producers (Neill 1988*b*; MacKay et al. 1990; Sandgren and Lehman 1990; Lehman and Cáceres 1993). Studies showing reciprocal relationships between *Daphnia*

© 1999 NRC Canada

and *Bythotrephes* abundance did not produce significant increases in algal biomass when *Daphnia* densities were low (Sandgren and Lehman 1990; Lehman and Cáceres 1993). The same pattern was observed by MacKay et al. (1990). Despite significant reductions of the dominant grazer *Daphnia pulex*, no effect was observed on chlorophyll *a* concentrations between treatments with and without *Chaoborus*. However, Neill (1988*b*) showed that phytoplankton biomass increased as a result of the presence of the invertebrate planktivore *Chaoborus*. The effect was only observed in highly productive systems where increases in microzooplankton production allowed *Chaoborus* to survive early instars and become abundant enough to efficiently reduce grazing macrozooplankton (Neill 1988*b*). This suggests that predation from invertebrate planktivores may affect primary producers if invertebrate planktivore density is sufficiently high for sufficiently long periods.

The effects that we observed on zooplankton size structure and abundance mainly occurred in the 40B and 60B treatments. In the 6B treatment, *Bythotrephes* was able to reduce *Bosmina* densities, whereas only minor effects were observed on *Holopedium* density and size. Comparing the densities of *Bythotrephes* used in our experiment with those observed in lakes with fish includes observations of *Bythotrephes cederstroemi* densities of over 100 individuals·m^{-3} (Berg and Garton 1988). This may be an extreme observation, but densities of about 10 individuals·m^{-3} or more have frequently been observed (Sprules et al. 1990; Hall and Yan 1997; Yan and Pawson 1997). Most of these observational studies also suggest effects of *Bythotrephes* predation on zooplankton community structure, although experimental data to confirm the observations are lacking. Our experiment thus sustains the hypothesis that the invertebrate planktivore *Bythotrephes* may exert a significant effect on zooplankton community structure and abundance in natural lake systems.

Acknowledgements

We thank Stefan Andersson and Thomas Mårtensson for help with the experiment. Helpful comments on earlier drafts of this manuscript were given by Lennart Persson, Pär Byström, Bent Christensen, and an anonymous reviewer. The research was supported by grants from the Helge Ax:on Johnsson foundation to E. Wahlström.

References

Allan, J.D. 1973. Competition and the relative abundances of two cladocerans. Ecology, **54**: 484–498.

Berg, D.J., and Garton, D.W. 1988. Seasonal abundance of the exotic predatory cladoceran, *Bythotrephes cederstroemi*, in western Lake Erie. J. Gt. Lakes Res. **14**: 479–488.

Black, R.W., II, and Hairston, N.G., Jr. 1988. Predator driven changes in community structure. Oecologia, **77**: 468–479.

Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Hertzig, A., Hillbricht-Ilkowska, A., Kurasawa, H., Larsson, P., and Weglenska, T. 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. **24**: 419–456.

Branstrator, D.K., and Lehman, J.T. 1991. Invertebrate predation in Lake Michigan: regulation of *Bosmina longirostris* by *Leptodora kinditii*. Limnol. Oceanogr. **36**: 483–495.

Bur, M.T., and Klarer, D.M. 1991. Prey selection for the exotic cladoceran *Bythotrephes cederstroemi* by selected Lake Erie fishes. J. Gt. Lakes Res. **17**: 85–93.

Burkhardt, S., and Lehman, J.T. 1994. Prey consumption and predatory effects of an invertebrate predator (*Bythotrephes*, Cladocera, Cercopagidae) based on phosphorous budgets. Limnol. Oceanogr. **39**: 1007–1019.

Elser, M.M., von Ende, C.N., Sorrano, P., and Carpenter, S.R. 1987. *Chaoborus* populations: response to food web manipulation and potential effects on zooplankton communities. Can. J. Zool. **65**: 2846–2852.

Gliwicz, Z.M., and Pijanowska, J. 1989. The role of predation in zooplankton succession. *In* Plankton ecology. *Edited by* U. Sommer. Springer-Verlag, Berlin. pp. 253–296.

Hall, D.J., Threlkeld, S.T., Burns, C.W., and Crowley, P.H. 1976. The size-efficiency hypothesis and the size structure of zooplankton communities. Annu. Rev. Ecol. Syst. **7**: 177–208.

Hall, R.I., and Yan, N.D. 1997. Comparing annual population growth estimates of the exotic invader *Bythotrephes* by using sediment and plankton records. Limnol. Oceanogr. **42**: 112–120.

Hamilton, J.D. 1958. On the biology of *Holopedium gibberum* Zaddach (Crustacea, Cladocera). Verh. Int. Ver. Limnol. **13**: 785–788.

Hanazato, T., and Yasuno, M. 1989. Zooplankton community structure driven by vertebrate and invertebrate predators. Oecologia, **81**: 450–458.

Hansson, L.-A., and Tranvik, L.J. 1996. Quantification of invertebrate predation and herbivory in food chains of low complexity. Oecologia, **108**: 542–551.

Lazzaro, X., Drenner, R.W., Stein, R.A., and Smith, J.D. 1992. Planktivores and plankton dynamics: effects of fish biomass and planktivore type. Can. J. Fish. Aquat. Sci. **49**: 1466–1473.

Lehman, J.T. 1991. Causes and consequences of cladoceran dynamics in Lake Michigan: implications of species invasion by *Bythotrephes*. J. Gt. Lakes Res. **17**: 437–445.

Lehman, J.T., and Branstrator, D.K. 1995. A model for growth, development, and diet selection by the invertebrate predator *Bythotrephes cederstroemi*. J. Gt. Lakes Res. **21**: 610–619.

Lehman, J.T., and Cáceres, C.E. 1993. Food-web responses to species invasion by a predatory invertebrate: *Bythotrephes* in Lake Michigan. Limnol. Oceanogr. **38**: 879–891.

Lunte, C.C., and Luecke, C. 1990. Trophic interactions of *Leptodora* in Lake Mendota. Limnol. Oceanogr. **35**: 1091–1100.

MacKay, N.A., Carpenter, S.R., and Soranno, P.A. 1990. The impact of two *Chaoborus* species on zooplankton community. Can. J. Zool. **68**: 981–985.

Makarewicz, J.C., Bertram, P., Lewis, T., and Brown, E.H., Jr. 1995. A decade of predatory control of zooplankton species composition of Lake Michigan. J. Gt. Lakes Res. **21**: 620–640.

Mordukhai-Boltovskaia, E.D. 1958. Preliminary notes on the feeding of the carnivorous cladocerans *Leptodora kinditii* and *Bythotrephes*. Dokl. Akad. Sci. SSSR, **112**: 113–1135.

Neill, W.E. 1988*a*. Complex interactions in oligotrophic lake food webs: responses to nutrient enrichment. *In* Complex interactions in lake communities. *Edited by* S.R. Carpenter. Springer-Verlag, New York. pp. 31–44.

Neill, W.E. 1988*b*. Community responses to experimental nutrient perturbations in oligotrophic lakes: the importance of bottlenecks in size-structured populations. *In* Size-structured populations. *Edited by* B. Ebenman and L. Persson. Springer-Verlag, Berlin. pp. 236–255.

Persson, L., Andersson, J., Wahlström, E., and Eklöv, P. 1996. Size-specific interactions in lake systems: predator gape limitation and prey growth rate and mortality. Ecology, **77**: 900–911.

© 1999 NRC Canada

Riessen, H.P., Sommerville, J.W., Chiappari, C., and Gustafson, D. 1988. *Chaoborus* predation, prey vulnerability, and their effect in zooplankton communities. Can. J. Fish. Aquat. Sci. **45**: 1912–1920.

Rodríguez, M.A., Magnan, P., and Lacasse, S. 1993. Fish species composition and lake abiotic variables in relation to the abundance and size structure of cladoceran zooplankton. Can. J. Fish. Aquat. Sci. **50**: 638–647.

Sandgren, C.D., and Lehman, J.T. 1990. Response of chlorophyll-a, phytoplankton, and microzooplankton to the invasion of Lake Michigan by *Bythotrephes*. Verh. Int. Ver. Limnol. **24**: 386–392.

Sprules, W.G., Riessen, H.P., and Jin, E.H. 1990. Dynamics of the *Bythotrephes* invasion of St. Lawrence Great Lakes. J. Gt. lakes Res. **16**: 346–351.

Vanderploeg, H.A., Liebig, J.R., and Omair, M. 1993. *Bythotrephes* predation on Great Lakes' zooplankton measured by an in situ method: implications for zooplankton community structure. Arch. Hydrobiol. **127**: 1–8.

Vanni, M.J. 1988. Freshwater zooplankton community structure: introduction of large invertebrate predators and large herbivores to a small-species community. Can. J. Fish. Aquat. Sci. **45**: 1758–1770.

Vinyard, G.L., and Menger, R.A. 1980. *Chaoborus americanus* predation on various zooplankters; functional response and behavioural observations. Oecologia, **45**: 90–93.

Wallace, R.L., and Snell, T.W. 1991. Rotifera. *In* Ecology and classification of North American freshwater invertebrates. *Edited by* J.H. Thorp and A.P. Covich. Academic Press, New York. pp. 187–248.

Yan, N.D., and Pawson, T.W. 1997. Changes in the crustacean zooplankton community of Harp Lake, Canada, following invasion by *Bythotrephes cederstroemi*. Freshwater Biol. **37**:409–425.

Yurista, P.M., and Schulz, K.L. 1995. Bioenergetic analysis of prey consumption by *Bythotrephes cederstroemi* in Lake Michigan. Can. J. Fish. Aquat. Sci. **52**: 141–150.

Zaret, T.M. 1980. Predation and freshwater communities. Yale University Press, New Haven, Conn.

© 1999 NRC Canada

Mixing and migration of overwintering Atlantic cod (*Gadus morhua*) stocks near the mouth of the Gulf of St. Lawrence

S.E. Campana, G.A. Chouinard, J.M. Hanson, and A. Fréchet

Abstract: Millions of Atlantic cod (*Gadus morhua*) migrate distances of up to 500 km each fall to aggregate together in a small overwintering area off eastern Canada. Synoptic research vessel surveys carried out each January between 1994 and 1997 documented dense aggregations of cod along both flanks of the Laurentian Channel in each year, with estimated biomasses exceeding 100 000 metric tons. Using the trace element composition of the otolith ("otolith elemental fingerprint") as a natural tag, we found members of four populations to be present on the overwintering grounds in significant numbers, yet large-scale mixing among the populations was minimal. Individual trawl samples were often composed of a single population, suggesting that population integrity was maintained at a scale of <20 km. Cod from the southern Gulf of St. Lawrence dominated the population composition along the southern flank of the Channel, while cod from the northern Gulf dominated the northern flank; the distributions of both of these populations extended well to the east of their summer habitats and were remarkably similar across years. There was no evidence of large-scale mixing across the Channel. In light of the substantive migration of northern Gulf cod into the management area for the southern Newfoundland population, fishing effort off southern Newfoundland has the potential to reduce the size of the northern Gulf population.

Résumé : Chaque automne, des millions de morues (*Gadus morhua*) parcourent des distances pouvant atteindre 500 km pour se regrouper dans une petite aire d'hivernage située au large de l'est du Canada. Des relevés synoptiques effectués par des navires de recherche océanographique au mois de janvier de chaque année, entre 1994 et 1997, ont documenté des agrégats denses de morue sur chacun des flancs du chenal Laurentien, les biomasses étant estimées à plus de 100 000 tonnes métriques. À l'aide de la composition en oligo-éléments de l'otolithe (« empreinte élémentaire de l'otolithe ») comme marque naturelle, nous avons observé la présence d'un certain nombre d'individus de quatre populations dans l'aire d'hivernage, bien qu'il y ait peu de mélange à grande échelle entre les populations. Les échantillons prélevés par les chaluts étaient souvent composés d'individus d'une seule population, ce qui indique que l'intégrité de la population serait maintenue à une échelle <20 km. La morue du sud du golfe du Saint-Laurent dominait la composition de la population sur le flanc méridional du Chenal, tandis que la morue du nord du Golfe dominait sur le flanc nord; les distributions de ces deux populations s'étendaient nettement à l'est de leurs habitats d'été, et elles étaient remarquablement similaires d'une année à l'autre. On n'a relevé aucune indication de mélange à grande échelle dans le Chenal. Compte tenu de la migration importante de la morue du nord du Golfe dans la zone de gestion visant la population du sud de Terre-Neuve, il est possible que l'effort de pêche mené au large de la partie sud de Terre-Neuve réduise la taille de la population de la partie nord du Golfe.

[Traduit par la Rédaction]

Introduction

The migration patterns of marine fish populations are less understood than the spawning migrations of anadromous fish such as salmon, but the influence on their population dynamics may be just as important. In response to spawning, feeding, or predator avoidance cues, marine fish migrations can extend over scales as small as a few kilometres in the case of flatfish moving offshore to overwinter (MacDonald et al. 1984) to thousands of kilometres for bluefin tuna (*Thunnus thynnus*) crossing the North Atlantic (Lutcavage et al. 1999). The extent and timing of these often-annual migrations are generally both consistent and predictable within a given population, suggesting an evolutionary advantage. However, the exact nature of the survival advantage, as well as the migratory route and destination, is often poorly under-

Received November 25, 1998. Accepted May 19, 1999.
J14919

S.E. Campana.[1] Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada.
G.A. Chouinard and J.M. Hanson. Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, NB E1C 9B6, Canada.
A. Fréchet. Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000, Mont Joli, QC G5H 3Z4, Canada.

[1]Author to whom all correspondence should be addressed. e-mail: campanas@mar.dfo-mpo.gc.ca

© 1999 NRC Canada

Table 1. Summary of Atlantic cod samples collected by area and season.

Area	Season	Date(s)	*n*	Length (cm)		Age (years)	
				Mean	SD	Mean	SD
North Gulf	Spring 1996	Apr. 13–30	155	56.5	10.9	7.6	2.1
	Spring 1997	Apr. 6–30	134	52.9	9.9	6.9	1.8
Southern Newfoundland, offshore	Spring 1996	Apr. 27–29	70	61.4	8.4	6.4	1.0
Southern Newfoundland, inshore (Fortune)	Spring 1997	June 19	42	64.6	15.4	—	—
Southern Newfoundland, inshore (Placentia)	Spring 1997	June 26	61	57.3	6.1	—	—
Southern Gulf	Spring 1996	June 13–15	200	52.1	7.9	7.3	1.4
	Spring 1997	June 11–13	200	53.4	6.1	7.6	1.5
Eastern Shelf (Gully)	Spring 1996	May 1	99	53.0	5.6	6.3	1.3
	Spring 1997	May 2	81	41.3	4.0	4.1	1.1
Northern Shelf (Banquereau)	Spring 1997	May 1	69	42.8	7.8	4.5	1.3
Cabot Strait	Winter 1996	Jan. 3–22	781	46.0	8.0	6.0	1.7
	Winter 1997	Jan. 6–25	866	45.5	9.3	5.8	1.8

Note: All spring samples were collected from spawning grounds around the time of spawning and thus represent populations of known identity. Winter samples of unknown population identity were subsequently classified using the spring samples as the reference.

stood, in part due to the technical limitations associated with tracking the movements of millions of fish deep beneath the ocean's surface.

Shortly after spawning in late spring, several Atlantic cod (*Gadus morhua*) populations off eastern Canada disperse to feed throughout the northern and southern Gulf of St. Lawrence, southern Newfoundland (NF), and the eastern Scotian Shelf. During the autumn, these same populations migrate to the warm deep waters of the Laurentian Channel (Cabot Strait) to overwinter, presumably to avoid near-freezing water temperatures and ice cover in their summer habitat (Templeman 1962; Jean 1964; Fréchet 1990). Cod density is high on the overwintering grounds, since the area occupied is about 10% of that occupied during the summer. However, particularly dense aggregations have been documented at various locations along both the northern and southern flanks of the Laurentian Channel (Templeman 1962; Jean 1964; Chouinard[2]). Although genetic (Bentzen et al. 1996) and tagging (Taggart et al. 1995) studies have both suggested the presence of four to six populations in the overwintering area, the spatial distribution of the populations and the extent of any population mixing are unknown. It seems unlikely that the 400–500 m depth of the Laurentian Channel is a physical impediment to cross-channel migration during the winter. Given that a substantial proportion of the biomass of the potential source populations is believed to overwinter in the area (Chouinard[2]), the likelihood of population mixing would appear to be high. Both mixed-population catches and distributions of populations outside management units can introduce serious error into the stock assessment of any fish species, thus degrading efforts at optimal management (Wood et al. 1989).

Tagging and genetic studies carried out to date lack the resolving power necessary to map the spatial extent of population mixing in the overwintering area. Therefore, we used the trace element composition of the otolith as a more highly resolved natural tag. The otolith is well suited to this application because it grows without resorption throughout the life of the fish (Campana and Neilson 1985) and it accretes trace elements from the environment in a temperature-sensitive manner (Kalish 1989; Edmonds et al. 1995; Fowler et al. 1995). Thus the elemental composition of the whole otolith ("otolith elemental fingerprint") serves as an environmentally induced natural tag of fish that have spent at least part of their lives in different habitats, independent of genetic identity. Recent studies have demonstrated that the elemental fingerprint of cod is not only population specific but that otolith growth and environmental shifts over periods of up to a year have a negligible effect on the fingerprint (Campana et al. 1995, 1999). By using the otolith elemental fingerprint of spawning cod on spawning grounds as a short-term natural tag, we were able to confidently track and identify those same groups of fish on the overwintering grounds with no more assumptions than those associated with genetic markers (Campana et al. 1999).

In this paper, we use the results of 4 years of synoptic research vessel surveys to document the distribution and density of cod on the overwintering grounds near the mouth of the Gulf of St. Lawrence. We then apply otolith elemental fingerprints as natural tags to provide the first description of population structure, distribution, and mixing within the overwintering area and assess the implications of the findings for migration theory and fisheries management.

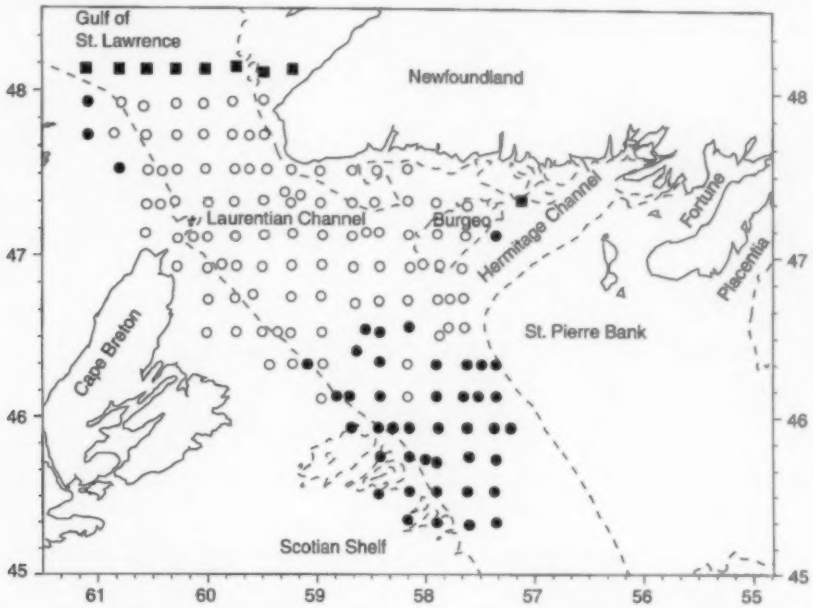
Materials and methods

A grid sampling design was used to survey the density and distribution of Atlantic cod in and around the approaches to the Gulf of St. Lawrence each January between 1994 and 1997. Surveys were carried out January 13–25, 1994, on the *Alfred Needler* (N197) (southern Gulf), January 8–28, 1994, on the *Gadus Atlantica* (G239) (northern Gulf), January 10–29, 1995, on the *Alfred Needler* (N214), January 3–22, 1996, on the *Wilfred Templeman* (WT182), and January 6–25, 1997, on the *Wilfred Templeman* (WT201). The nominal distance between survey samples was 20 km, although some areas were surveyed at 5-km intervals. Survey biomass (minimum trawlable biomass) was calculated by multiplying the mean weight per standardized tow by the number of trawlable units in the survey area. The resulting biomass distribu-

[2] Chouinard, G.A. Unpublished manuscript. Distribution of Atlantic cod (*Gadus morhua*) in Cabot Strait during January 1996 and 1997. Workshop on the Identification of Cod Stocks, Dartmouth, N.S.

© 1999 NRC Canada

Fig. 1. Map of the study area showing the Atlantic cod overwintering grounds sampled in January 1996 (●), January 1997 (■), and January of both years (○). The 200-m contour is shown.



tions were contoured using Delauney triangles in the spatial analysis program ACON.

Otoliths for determination of stock identity were collected from adult cod (35–85 cm) on approximately every second set of the 1996 and 1997 surveys (Table 1; Fig. 1). A total of 138 successful sets were made in 1996, of which 60 sets were sampled for otoliths (n = 754). Samples from 1997 came from 104 successful sets, of which 49 sets were sampled for otoliths (n = 866). Additional samples (n = 89) (45°50′ latitude, 58°03′ longitude) to the east of the region surveyed by the *Wilfred Templeman* in 1997 were collected on March 15, 1997, in two sets as part of the *Alfred Needler* N255 spring survey.

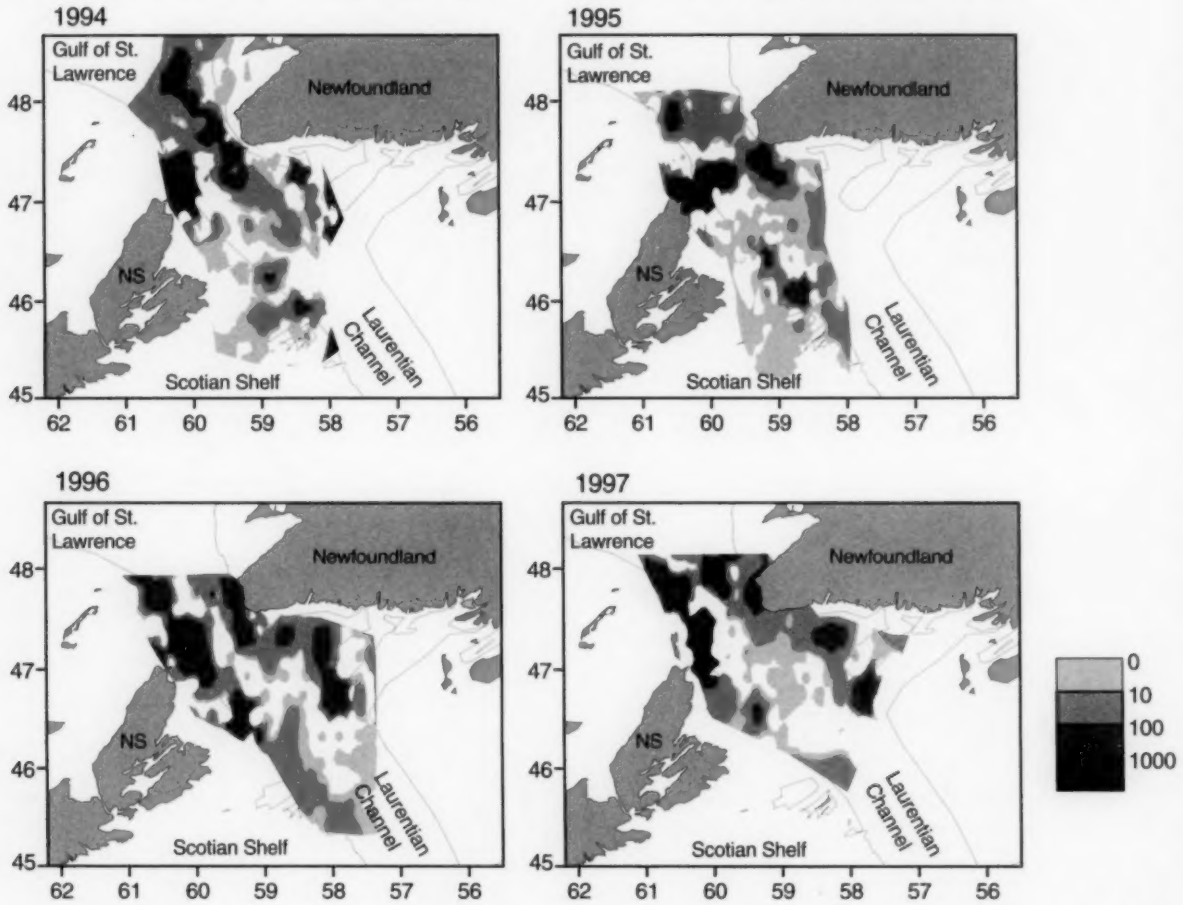
Each cod population off the eastern coast of Canada spawns at a distinct site and is thus of known population identity at the time of spawning (Templeman 1962). Since the otolith elemental fingerprints of the 1996 and 1997 spring-spawning aggregations differed significantly among spawning groups (Campana et al. 1999), they were used as population- and year-specific reference markers against which the winter samples could be compared (Table 1). The elemental fingerprint of each otolith was characterized by a suite of five elements (Li, Mg, Mn, Sr, Ba) using isotope dilution inductively coupled plasma mass spectrometry and procedures described in Campana et al. (1999). Elemental assays for both spring spawner (known identity) and winter (unknown identity) samples from each year were systematically randomized with respect to assay sequence and analyzed together at the same laboratory. As a result, spring spawner and winter samples from any given year are completely comparable. To insure that differences in fish length or age among samples were not confused with population-specific differences, the effect of otolith weight was statistically removed from those elemental concentrations in which a size effect was evident (Campana et al. 1999). Detrended elemental concentrations showed no obvious residual relationship with fish size (and by inference, fish age) for most of the sample collections. In any event, relative differences among detrended samples tended to be very similar to those based on original data, with the exception of samples of very small fish. Since small intercalibration differences between laboratories could have introduced artifactual differences among years, only spring spawner samples collected in the same year as that of the January survey were used in classification. Thus the January 1996 samples were classified as to population completely independently and using a different set of reference samples than those in 1997. A second set of 1997 classifications was carried out using the 1997 spring spawner reference samples in conjunction with the 1996 spring spawner sample from St. Pierre Bank, since the latter area was not sampled in 1997. Tests of classification accuracy were also made against collections of cod made throughout the Gulf of St. Lawrence in the fall of 1995 (Campana et al. 1999).

Stock composition analysis of each set in the winter samples was carried out using a maximum likelihood based analysis analogous to that of Millar's (1990) HISEAS program. The reference data for all elemental fingerprints were the spring spawner fingerprints from the same year, for which stock affinity was known. Conceptually, the stock composition analysis used the statistical distribution of the elemental fingerprints in each reference (spawning) group to estimate the proportion of each reference population in the unknown (winter) mixture. As long as all of the possible groups contributing to the group mixture have been characterized, the analysis will produce an unbiased estimate of population proportions, with no more assumptions than those associated with genetic markers (Wood et al. 1987, 1989; Campana et al. 1999). The product of the analysis was the proportion of each reference population in each set, which was subsequently converted to population-specific biomass as described earlier. Unsampled sets were assumed to contain the same population proportions as the nearest neighbouring set along a similar depth contour.

Population-specific biomass estimates from the winter surveys were subsequently compared with those from summer and fall research vessel surveys, which is a time when populations are largely separated. Biomass (all ages) calculations from those surveys were calculated as the mean of the preceding and following years, so as to approximate the biomass in January. Biomass estimates for the northern Gulf population were calculated as the mean of the sum-

© 1999 NRC Canada

Fig. 2. Relative Atlantic cod abundance on overwintering grounds near the mouth of the Gulf of St. Lawrence off eastern Canada as determined with bottom trawl surveys in January of 1994–1997. The densest aggregations were consistently found in the same locations along each flank of the Laurentian Channel. Estimated biomass in each year was between 100 000 and 200 000 tonnes. The 1994 data represent the results of two separate surveys. NS = Nova Scotia. The 200-m bathymetric contour is shown.



mer and fall mobile gear sentinel surveys in the preceding and following years. The estimate for the southern NF population was based on the April 1996 survey. All biomass estimates represent minimum trawlable biomass, with no adjustment for catchability.

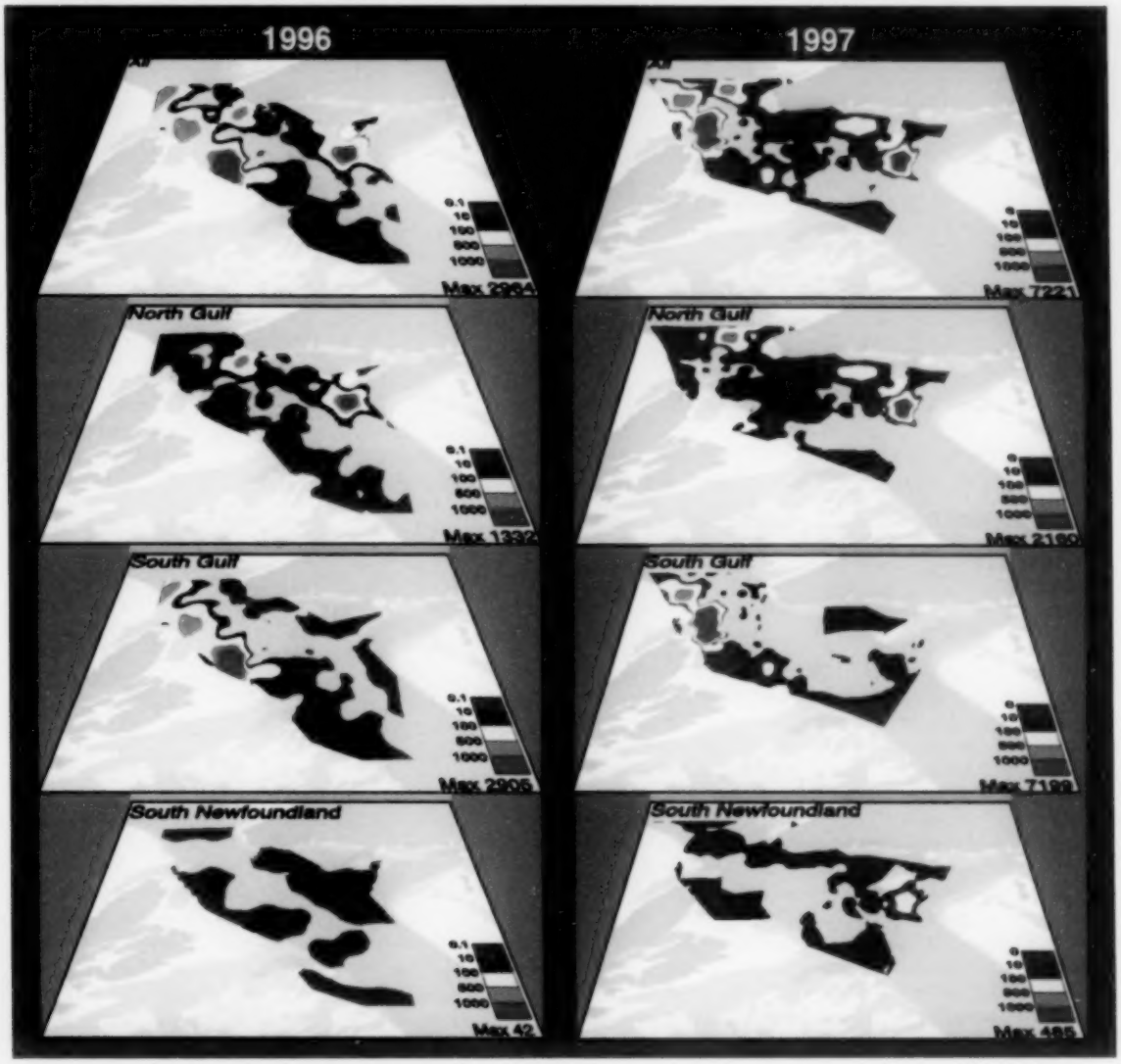
Results

January trawl surveys of the overwintering area documented dense aggregations of Atlantic cod at 250–450 m depth in various locations along the northern and southern flanks of the Laurentian Channel (Fig. 2). The locations of these aggregations were remarkably consistent from year to year, as were the relatively low densities in the centre of the Channel. Water temperature at the sites of large aggregations generally ranged between 4.9 and 5.3°C.

The accuracy and precision of the population identifications based on the otolith elemental fingerprints appeared to be high and were not a source of important error. When the spring-spawning reference samples of 1996 (four populations) were used to classify cod collected in the southern and northern Gulf in the fall of 1995 (which were probably in their own stock area at the time), 98.9% of the cod captured in the southern Gulf were identified as being of southern Gulf origin, while 99.2% of the cod captured in the northern Gulf were identified as being of northern Gulf origin. To the extent that the southern and northern Gulf cod avoided mixing between the fall of 1995 and the spring of 1996, these results suggest that these two groups of fish were easily differentiated. The results of the second test of accuracy were based on classification of the 1996 spring-spawning samples using the 1997 spawners (seven spawning groups) as the reference (Table 1). Classification accuracy for the northern Gulf, southern Gulf, and Scotian Shelf (Gully) spawning groups was 77, 89, and 70%, respectively. Misclassification error for the Scotian Shelf group was primarily associated with southern Newfoundland spawners, indicating that their elemental fingerprints were similar. Accuracy increased to more than 96% for both the northern Gulf and southern Gulf groups when the 1996 spawners (four reference groups) were used to classify the 1997 spawners. The accuracy of all

© 1999 NRC Canada

Fig. 3. Population-specific abundance of adult Atlantic cod on overwintering grounds near the mouth of the Gulf of St. Lawrence in January of 1996 and 1997. The top layer shows the overall cod abundance in each year, while each of the following layers shows the abundance attributable to the specific population named on the layer. Total abundance was decomposed into component populations through analysis of otolith elemental fingerprints, which served as natural tags for each of the component populations.

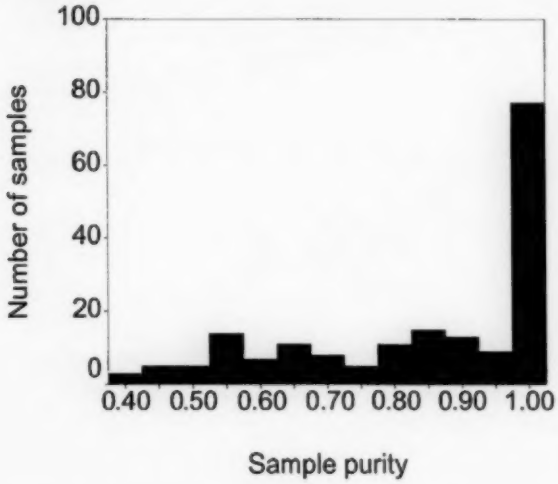


of these tests would be expected to increase as the interval between sampling reference and test groups was reduced, due to the reduced opportunity for otolith growth between the time of initial characterization and testing. Since the interval used in the identification of the winter samples was half of that used above, it would appear that a coefficient of variation of 5–20% is likely in the analyses that follow.

On the basis of the stock composition analysis of the individual trawl samples, it was possible to contour population-specific abundance to show relative abundance and distribution each winter (Fig. 3). The population-specific distribution patterns in the two years were very similar and indicated that the two largest populations in the area remained largely separate in their distribution. Southern Gulf cod dominated the aggregations on the southern flank of the Laurentian Channel, while northern Gulf cod dominated aggregations on the northern flank and in the central part of the Channel. There was no evidence of large-scale mixing across the Channel, although northern Gulf cod were more prevalent in the centre of the Channel than were southern Gulf cod. The overall representation of the population from offshore southern Newfoundland (St. Pierre Bank) was relatively low and restricted to the area immediately adjacent to St. Pierre Bank. On the other hand, two other populations from inshore southern Newfoundland (Fortune Bay and Placentia Bay), as well as that from the eastern Scotian

© 1999 NRC Canada

Fig. 4. Purity of each bottom trawl sample in terms of Atlantic cod population composition. The population mixture analysis indicated that four populations contributed significantly to the overwintering grounds; hence, the estimated proportion of the dominant population in each sample should vary randomly between 0.25 and 1.00. Instead, 69% of the 183 samples consisted of essentially only one population (>80% purity), indicating that the populations did not intermix on a local scale. Both 1996 and 1997 samples where the catch was ≥10 fish are presented.



Shelf, were not present in significant numbers (< 5% of total numbers) anywhere in the survey area. The poor representation of the eastern Scotian Shelf population may have been amplified by its extremely low current population size, although all of the characterized populations except those from southern Newfoundland are thought to be at low abundance levels.

The segregation among populations occurred on a scale even finer than that indicated by the contoured population distributions. Samples from a single tow were generally dominated by a single population and, in 69% of samples containing at least 10 individuals, were essentially pure (Fig. 4). This was the case even where adjacent samples were characterized by completely different population compositions. Allowing for unavoidable integration of closely spaced schools across the 2-km tow length, the overall purity of the samples suggests that population integrity was maintained at a scale of < 20 km, corresponding to the mean distance between sites in the sampling grid. The one area in which true population mixing may have occurred is the Hermitage Channel to the west of St. Pierre Bank. There, both the northern Gulf and southern Newfoundland populations were present in significant numbers, but we were unable to determine if individual schools were pure in composition but tightly packed or of mixed composition.

Minimum trawlable biomass in the 1996 and 1997 winter surveys was estimated at 102 800 and 134 200 tonnes (metric tons), respectively, which is a substantial proportion of the biomass of the potential source populations (Table 2). When calculated on a population-specific basis, the northern Gulf and southern Gulf populations together comprised more than 91% of the biomass of the surveyed region in each year, with the rest being made up of cod from the remaining populations. Population-specific biomass estimates differed relatively little between years for the northern Gulf, southern Newfoundland, and Scotian Shelf populations. The interyear difference was more marked for the southern Gulf population; however, much of this difference was attributable to greater survey coverage of the southern Gulf in 1997. Comparisons with biomass estimates made in summer and fall, at a time when the various populations were largely separated, suggested that a large proportion of the southern and northern Gulf populations were present in the survey region during the winter (Table 2). In contrast, < 20% of the southern Newfoundland and Scotian Shelf populations were present in the survey region, suggesting that their overwintering grounds were elsewhere.

The distribution of population-specific biomass was not in keeping with current stock management units (Table 2). In particular, less than half of the northern Gulf biomass was determined to be present in the northern Gulf management unit during the winter, with most of the remainder being found well inside the boundaries of the southern Newfoundland region, mixed with the resident fish. This pattern was similar in both years. Similarly, <13% of the cod collected in the surveyed region of the Scotian Shelf were actually of Scotian Shelf origin, with most of the remainder (74% in 1996; 83% in 1997) being from the southern Gulf. In contrast, the portion of the southern Newfoundland population that was surveyed seemed to be largely restricted to the southern Newfoundland management unit (NAFO Division 3Ps), and >91% of the southern Gulf population was found in the appropriate management unit (4TVn, November–April). Very few differences in biomass distribution were evident between the years.

Discussion

It has been known for some time that the approaches and mouth of the Gulf of St. Lawrence serve as the overwintering grounds for several large Atlantic cod populations in the area (Templeman 1962; Jean 1964). However, the distribution of cod on the overwintering grounds was not evident until the detailed winter surveys reported in this study had been completed. Over the 4 years of surveys, cod showed remarkable year-to-year consistency in the location of their overwintering aggregations along the slopes of the Laurentian Channel, suggesting that some feature of the bottom topography or environment made the location a preferred habitat. Preferred depths tended to be close to the mean depth of the Channel (400–500 m), suggesting that depth was not a physical impediment to cross-channel migration during the winter. Nor did distribution appear to be a function of water temperature alone, since the preferred temperature of 4.9–5.3°C was widely available in the Laurentian Channel (Swain et al. 1998). Other fish species also migrate out of the Gulf each winter (Swain et al. 1998), suggesting that the ecological or evolutionary motivation for migration is broadly shared.

© 1999 NRC Canada

Table 2. Population-specific Atlantic cod biomass (tonnes) from the January 1996 and 1997 research vessel surveys in each of the management areas based on classification of the otolith elemental fingerprints.

	1996 reference population				
January 1996 area of capture	Southern Newfoundland	Northern Gulf	Southern Gulf	Scotian Shelf (Gully)	Total
Southern Newfoundland	3 269	23 955	516	542	28 643
Northern Gulf	870	12 519	2 145	798	16 330
Southern Gulf	0	1 018	5 845	368	7 230
Cape Breton (4Vn)	107	4 440	43 028	950	48 525
Scotian Shelf	140	222	1 152	153	1 668
Total	4 746	42 154	52 685	2 810	102 800
Biomass from summer surveys	41 100	34 500	88 000	14 000	

	1997 reference population							
	Southern Newfoundland					Scotian Shelf		
January 1997 area of capture	St. Pierre Bank	Fortune	Placentia	Northern Gulf	Southern Gulf	Banquereau	Gully	Total
Southern Newfoundland	5 882	93	2237	24 396	45	0	273	32 963
Northern Gulf	655	115	2	23 061	92	0	549	23 960
Southern Gulf	38	0	0	923	28 547	1037	0	30 549
Cape Breton (4Vn)	263	1	0	1 288	43 898	417	18	45 880
Total	6 839	209	2238	49 801	72 583	1454	840	133 900
Biomass from summer surveys	----------41 100----------			43 000	86 000	----------7800----------		

Note: Mean population-specific biomass estimates from the the summer–fall surveys of the preceding and following years are tabled for comparison. The biomass for 3Ps cod is from the April 1996 research vessel survey. Discrepancies between the total survey biomass and the sum of the population-specific biomasses are due to rounding errors during classification.

Our results indicate that four major cod populations (northern Gulf, southern Gulf, southern Newfoundland, and eastern Scotian Shelf) contributed fish to the overwintering grounds in the Laurentian Channel, dominated by contributions from the northern and southern Gulf populations. Although the presence of these populations had previously been suspected based on otolith elemental fingerprints (Campana et al. 1999), genetics (Bentzen et al. 1996), and tagging studies (Taggart et al. 1995), the location and relative contribution of the populations in winter were unknown. Historic tagging studies suggested that small numbers of an additional population (Cape Breton cod, 4Vn) may also have been present (McKenzie 1956). However, we could not address that issue because the elemental fingerprints of spawning fish sampled from the Cape Breton area were not distinguishable from those of the southern Gulf (Campana et al. 1999).

The otolith elemental fingerprints used in this study performed well as natural tags and provided population-specific distributions with a resolving power that was simply not possible in previous tagging and genetic studies. Our findings indicated that Gulf cod overwinter along the flanks of the Laurentian Channel, resulting in population extensions well to the east of their summer habitats. In general, these findings reinforce expectations based on tagging studies (McKenzie 1956; Taggart et al. 1995), fleet dynamics (Sinclair and Currie 1994), research vessel surveys (Rollet et al. 1994), and analyses of size composition (Hanson 1995). In particular, the near absence of cross-channel migration is completely consistent with past tagging studies, which reported on average <5% movement across the channel from either direction (McKenzie 1956; Templeman 1962; Martin and Jean 1964; Taggart et al. 1995). Several other results came as a surprise, however. The first was the relative purity of the population composition in the individual survey tows, suggesting that population integrity was maintained at a scale of <20 km. With the exception of the area to the west of St. Pierre Bank, there was little evidence of population mixing either across or on any given side of the Channel. Such a degree of population segregation is remarkable given the 100- to 500-km migration distance of many of the individuals in the two Gulf populations. Few analogies exist in the marine groundfish literature, although the homing capabilities of Atlantic herring (*Clupea harengus*) and other pelagic fishes to spatially segregated spawning grounds are well documented (Wheeler and Winters 1984). In the only reported parallel, Rose (1993) used hydroacoustic gear to monitor a portion of a 250-km migration by a 500 000-tonnes school of cod from an offshore spawning ground to an inshore feeding area during which the integrity of the school was maintained. The migration pathway of the cod in the current study was not monitored. However, in light of the relationship between size of cod and distance from the mouth of the Gulf (Chouinard[2]), larger cod apparently migrated further out of the Gulf than did smaller cod, consistent with Rose's (1993) suggestion that older, more experienced cod lead the way for others to follow. Alternatively, larger cod may simply have migrated further, due to the energetic efficiencies of a larger body size (Dodson 1997). Tagging studies on cod in and around the Gulf of St. Lawrence indicate that homing to spring-spawning grounds after overwintering is carried out with relatively little loss to other spawning groups (Taggart et al. 1995). If migration to and from the overwintering grounds is indeed a learned response, it could be that spatial segregation of cod populations on the overwintering grounds is a behavioural mecha-

© 1999 NRC Canada

nism through which faithful returns to spawning grounds are maximized. Such a mechanism would presumably supplement or reinforce philopatry brought on by imprinting at an early life history stage (Cury 1994). However, the role of imprinting and learning in the development of migratory patterns remains unclear (Quinn and Brodeur 1991), particularly given the increasing evidence for innate directional mechanisms in many fishes (Walker et al. 1997).

A second unexpected result was the extent of the eastward distribution of both the southern and northern Gulf populations. The result indicating movement of southern Gulf cod as far east as Banquereau Bank on the eastern Scotian Shelf is consistent with previous reports (McKenzie 1956; Martin and Jean 1964; Sinclair and Currie 1994; Campana et al. 1995; Hanson 1995), although the relative dominance of the southern Gulf population had never been quantified in such detail. On the other hand, there was no prior suggestion that the winter distribution of northern Gulf cod extended so completely to the southeast of Burgeo Bank, despite having previously been noted as a stock mixing area (Templeman 1962; D'Amours et al. 1994; Rollet et al. 1994). The distance between Burgeo Bank and St. Pierre Bank is relatively small and is probably not meaningful in a biological context. However, the relative concentration of northern Gulf cod in this area was large, characterized by the highest densities of this population in both survey years. It is difficult to discount these findings as anomalies, given that the reconstructed population distributional patterns were virtually identical between years, despite being based on completely independent reference collections. Of course, historic distributional patterns may have differed from those present now, perhaps in response to different environmental conditions or abundance. However, the results of synoptic research survey distributions in the 1980s (Rollet et al. 1994) and historic and current tag returns as reported by Taggart et al. (1995) were completely consistent with our findings: the Hermitage Channel area to the west of St. Pierre Bank often served as the easternmost boundary for the northern Gulf migration, as well as the westernmost boundary for movements of more westerly groups of cod. Thus, there is no evidence to suggest that large-scale movement of northern Gulf cod to the Hermitage Channel is merely a recent phenomenon.

Substantive migration of northern Gulf cod into the southern Newfoundland management unit has significant management implications. With more than half of the northern Gulf population biomass present in the southern Newfoundland management unit during the winter, fishing effort in southern Newfoundland to the west of St. Pierre Bank has the potential to reduce the relative abundance of the northern Gulf population. Indeed, a given unit of fishing effort in this area in the winter of 1996 or 1997 would have caught three to four times more northern Gulf cod than resident southern Newfoundland cod. Maximal influence would be associated with fishing activity in the winter, since this is the period during which the largest numbers of northern Gulf cod are present in the management area for the southern Newfoundland population, but some effect would also be expected in the spring before the return migration of northern Gulf cod has been completed.

As noted by Shelton et al. (1996), the extension of northern Gulf cod into the southern Newfoundland area also poses problems for the interpretation of the winter research surveys previously used to estimate the abundance of the southern Newfoundland population. Current research surveys of the region are carried out in April, under the assumption that most northern Gulf cod have left the area. However, it is not clear that the return migration has necessarily been completed by April. Research survey abundance indices based on mixed populations would provide an inflated abundance estimate for the resident population.

While differences undoubtedly exist, the migration patterns of southern Gulf cod appear to parallel those of northern Gulf cod, making the southern flank of the Laurentian Channel biologically analogous to that of the northern flank. Along the southern flank, fisheries management has made accomodations for this migration pattern by acknowledging that fishing off Cape Breton (4Vn) and the eastern Scotian Shelf (4Vsb) in winter–spring is likely to catch high proportions of southern Gulf cod. Research surveys of the Scotian Shelf are carried out in July, long after the return migration to the Gulf has been completed, thus avoiding contamination of abundance indices due to the presence of southern Gulf cod. However, no such accomodations have yet been made on the northern edge of the Channel, despite the close analogy to the southern Gulf cod. Further examination of this issue appears warranted.

Acknowledgements

We thank B. Anderson, J. Brattey, P. Comeau, K. Drinkwater, P. Fanning, D. Gray, J. Hamel, R. Kean, F. Kelly, L. Marks, and S. Smith for their assistance with this project. Principle funding was provided by the Strategic Research Program of the Department of Fisheries and Oceans, with supplemental funding for the 1997 survey provided by Earthwatch and its Research Corps.

References

Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. **53**: 2706–2721.

Campana, S.E., and Neilson, J.D. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. **42**: 1014–1032.

Campana, S.E., Gagne, J.A., and McLaren, J.W. 1995. Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar. Ecol. Prog. Ser. **122**: 115–120.

Campana, S.E., Chouinard, G.A., Hanson, M., Fréchet, A., and Brattey, J. 1999. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. In press.

Cury, P. 1994. Obstinate nature: an ecology of individuals. Thoughts on reproductive behavior and biodiversity. Can. J. Fish. Aquat. Sci. **51**: 1664–1673.

D'Amours, D., Frank, K.T., and Bugden, G. 1994. Report of the Working Group on Oceanographic Effects on Stock Migration and Mixing — reviewed by the Fisheries Oceanography Committee (FOC). DFO Atl. Fish. Res. Doc. 94/54.

Dodson, J.J. 1997. Fish migration: an evolutionary perspective. *In* Behavioural ecology of fishes. *Edited by* J.-G.J. Godin. Oxford University Press, Oxford, U.K. pp. 10–36.

Edmonds, J.S., Caputi, N., Moran, M.J., Fletcher, W.J., and Morita, M. 1995. Population discrimination by variation in concentra-

© 1999 NRC Canada

tions of minor and trace elements in sagittae of two Western Australian teleosts. *In* Recent developments in fish otolith research. *Edited by* D.H. Secor, J.M. Dean, and S.E. Campana. University of South Carolina Press, Columbia, S.C. pp. 655–670.

Fowler, A.J., Campana, S.E., Jones, C.M., and Thorrold, S.R. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. Can. J. Fish. Aquat. Sci. **52**: 1421–1430.

Fréchet, A. 1990. Catchability variations of cod in the marginal ice zone. Can. J. Fish. Aquat. Sci. **47**: 1678–1683.

Hanson, J.M. 1995. Separation of catches of two Atlantic cod stocks in an otter trawl fishery by application of the EM algorithm. N. Am. J. Fish. Manage. **15**: 277–289.

Jean, Y. 1964. Seasonal distribution of cod (*Gadus morhua* L.) along the Canadian Atlantic coast in relation to water temperature. J. Fish. Res. Board Can. **21**: 429–460.

Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. **132**: 151–178.

Lutcavage, M.E., Brill, R.W., Skomal, G.B., Chase, B.C., and Howey, P.W. 1999. Results of pop-up satellite tagging of spawning size class fish in the Gulf of Maine: do North Atlantic bluefin tuna spawn in the mid-Atlantic? Can. J. Fish. Aquat. Sci. **56**: 173–177.

MacDonald, J.S., Dadswell, M.J., Appy, R.G., Melvin, G.D., and Methven, D.A. 1984. Fishes, fish assemblages, and their seasonal movements in the lower Bay of Fundy and Passamaquoddy Bay, Canada. Fish. Bull. U.S. **82**: 121–139.

Martin, W.R., and Jean, Y. 1964. Winter cod taggings off Cape Breton and on offshore Nova Scotia banks, 1959–1962. J. Fish. Res. Board Can. **21**: 215–238.

McKenzie, R.A. 1956. Atlantic cod tagging off the Southern Canadian mainland. Bull. Fish. Res. Board Can. **105**: 1–93.

Millar, R.B. 1990. Comparison of methods for estimating mixed stock fishery composition. Can. J. Fish. Aquat. Sci. **47**: 2235–2241.

Quinn, T.P., and Brodeur, R.D. 1991. Intra-specific variations in the movement patterns of marine animals. Am. Zool. **31**: 231–241.

Rollet, C., Fréchet, A., Battaglia, A., and Brethes, J.C. 1994. Modification de distribution du stock de morue du nord du golfe du Saint-Laurent (3Pn4RS) en hiver. DFO Atl. Fish. Res. Doc. 94/82.

Rose, G.A. 1993. Cod spawning on a migration highway in the north-west Atlantic. Nature (Lond.), **366**: 458–461.

Shelton, P.A., Stansbury, D.E., Murphy, E.F., Brattey, J., and Lilly, G.R. 1996. An assessment of the cod stock in NAFO Subdivision 3Ps. DFO Atl. Fish. Res. Doc. 96/91.

Sinclair, A.F., and Currie, L. 1994. Timing of commercial fisheries based on the movement of cod into and out of the Gulf of St. Lawrence, 1986–93. DFO Atl. Fish. Res. Doc. 94/47.

Swain, D.P., Chouinard, G.A., Morin, R., and Drinkwater, K.F. 1998. Seasonal variation in the habitat associations of Atlantic cod (*Gadus morhua*) and American plaice (*Hippoglossoides platessoides*) from the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **55**: 2548–2561.

Taggart, C.T., Penney, P., Barrowman, N., and George, C. 1995. The 1954–1993 Newfoundland cod-tagging database: statistical summaries and spatial–temporal distributions. Can. Tech. Rep. Fish. Aquat. Sci. No. 2042.

Templeman, W. 1962. Divisions of cod stocks in the Northwest Atlantic. ICNAF Redbook, III. pp. 79–123.

Walker, M.M., Diebel, C.E., Haugh, C.V., Pankhurst, P.M., Montgomery, J.C., and Green, C.R. 1997. Structure and function of the vertebrate magnetic sense. Nature (Lond.), **390**: 371–376.

Wheeler, J.P., and Winters, G.H. 1984. Homing of Atlantic herring (*Clupea harengus harengus*) in Newfoundland waters as indicated by tagging data. Can. J. Fish. Aquat. Sci. **41**: 108–117.

Wood, C.C., McKinnell, S., Mulligan, T.J., and Fournier, D.A. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. **44**: 866–881.

Wood, C.C., Rutherford, D.T., and McKinnell, S. 1989. Identification of sockeye salmon (*Oncorhynchus nerka*) stocks in mixed-stock fisheries in British Columbia and southeast Alaska using biological markers. Can. J. Fish. Aquat. Sci. **46**: 2108–2120.

© 1999 NRC Canada

Potential effects of maternal factors on spawning stock–recruitment relationships under varying fishing pressure

Beth Scott, Gudrun Marteinsdottir, and Peter Wright

Abstract: The use of spawning stock biomass as a direct measure of reproductive potential may not be valid because of age- or size-specific differences in fecundity and the effect of maternal size and condition on offspring viability. In this study, we examine the potential significance of these effects using modelled Atlantic cod (*Gadus morhua*) populations. We quantify how changes in the age composition of the spawning stock, due to a range of fishing pressures and under different stock–recruitment relationships, could influence the reproductive output. Quantitative comparisons were made between a "standard" population where all age-classes only suffer natural instantaneous mortality (M = 0.2) and populations that suffer increasing levels of fishing pressure (F = 0.0–1.0). The results of the modelling exercise suggests that if the effects of the loss of more fecund older/larger individuals in the population are not considered, the number of potential recruits produced by populations under higher levels of fishing mortality could be overestimated by as much as 60%. When age/size-related maternal effects on egg viability are also considered, the amount of potential recruits can be overestimated by a further 10% in the heavily exploited populations.

Résumé : L'utilisation de la biomasse du stock de géniteurs comme mesure directe du potentiel reproducteur pourrait ne pas être valable en raison des différences de fécondité liées à l'âge ou à la taille et de l'effet de la taille et de l'état des génitrices sur la viabilité de la progéniture. Dans la présente étude, nous examinons l'importance de ces effets dans des populations de morues modélisées. Nous établissons quantitativement comment les changements dans la composition par âge du stock de géniteurs, liés à des pressions de pêche diverses et sous différentes relations stock-recrutement, peuvent influer sur la reproduction. Nous avons effectué des comparaisons quantitatives entre une population « standard », dans laquelle toutes les classes d'âge ne subissent qu'une mortalité instantanée naturelle (M = 0,2), et des populations exposées à divers niveaux de pression de pêche (F = 0,0 à 1,0). Les résultats de la modélisation indiquent que si les effets de la perte des sujets plus âgés, plus gros et plus féconds dans la population ne sont pas considérés, le nombre de recrues potentiellement produites dans les populations sous les niveaux plus élevés de mortalité par pêche pourrait être surestimé d'un pourcentage pouvant atteindre 60%. Si, en outre, on tient compte des effets de l'âge et de la taille des génitrices sur la viabilité des oeufs, le nombre de recrues potentielles peut être surestimé de 10% de plus dans les populations fortement exploitées.

[Traduit par la Rédaction]

Introduction

The true nature of stock–recruitment relationships may be obscured by the use of spawning stock biomass as a direct measure of reproductive potential. This is because population fecundity (the total egg production for a given stock biomass) can vary due to changes in reproductive parameters such as age at first maturity (Stearns and Crandall 1984; Jorgensen 1990) and age- or size-specific fecundity (Hodder 1965; Hislop 1988; Kjesbu et al. 1991; Mackenzie et al. 1998; Marshall et al. 1998). In addition, there is now accumulating evidence that maternal size and condition may influence offspring viability, as it has been shown for Atlantic cod (*Gadus morhua*) that the size, age, and condition of females influence the size of eggs and larvae (Kjesbu 1989; Chambers and Waiwood 1996; Chambers 1997; Trippel et al. 1997; Marteinsdottir and Steinarsson 1998; Trippel 1998). The influence of female spawning experience on hatching success is demonstrated by lower hatching success among first-time spawners compared with second- or third-time spawners (Solemdal et al.1995; Trippel 1998). Positive correlations have been detected between female condition and larval feeding success and larval specific growth rates (Marteinsdottir and Steinarsson 1998). Additionally, larvae from large eggs, which are initially larger at hatching, have been shown to start feeding earlier, exhibit greater feeding success, have a greater tendency to develop functional swim bladders successfully, and exhibit higher rates of swimming activity and growth (Knutsen and Tilseth 1985; Solemdal et al. 1992; Marteinsdottir and Steinarsson 1998). All of these

Received December 21, 1998. Accepted June 18, 1999.
J14939

B. Scott.[1] Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB24 3QY, U.K., and Fisheries Research Services, Marine Laboratory Aberdeen, P.O. Box 101, Victoria Road, Aberdeen, AB11 9DB, U.K.
G. Marteinsdottir. Marine Research Institute, Skulagata 4, P.O. Box 1390, 121 Reykjavik, Iceland.
P. Wright. Fisheries Research Services, Marine Laboratory Aberdeen, P.O. Box 101, Victoria Road, Aberdeen, AB11 9DB, U.K.

[1]Author to whom all correspondence should be addressed.
e-mail: scottb@marlab.ac.uk

© 1999 NRC Canada

factors suggest that maternal factors influence viability such that older age-classes may have a disproportionate contribution to the number of successful recruits.

The level of fishing exploitation influences the age and size structures of spawning populations and can result in a severe depletion of spawners from the older age-classes (Trippel et al. 1997 and references therein). Excessive removal of the older and larger individuals from fish stocks may be more important to recruitment than changes in absolute spawning stock biomass. For example, recruitment in Icelandic cod was found to be influenced more by the age structure than by the size of the spawning population such that the stock–recruitment relationship was improved extensively by including information on age diversity of spawners (Marteinsdottir and Thorarinsson 1998). On the whole, maternal effects on viability have largely been overlooked by fisheries management, but they may account for significant uncertainty found in most stock–recruitment relationships (Mackenzie et al. 1998; Marshall et al. 1998).

Using life history parameters for Icelandic cod as our modelled multiple spawner, this study quantifies the relative potential impact of maternal factors on the production of recruits. Traditionally, the calculation of potential recruits from the recruitment versus spawning stock biomass relationships (Ricker 1954; Beverton and Holt 1957) is based on the assumptions that (*i*) there is a mean number of eggs produced per unit biomass and (*ii*) all offspring have equal survival probabilities. We investigated the effects of these two assumptions independently as well as in combination and over a wide range of realistic fishing pressures.

Materials and methods

To investigate the effects that differences in the mean number of eggs produced per unit biomass could have on potential reproductive output, we created two idealised scenarios that encompass two extremes of stock–recruitment relationships. In the first scenario, hereafter referred to as "constant biomass," all modelled populations maintain a constant spawning stock biomass with increasing fishing pressure by allowing the numbers of recruits to increase proportionally with decreases in the biomass of older age-classes. This scenario allows us to quantify the number of potential recruits produced, per unit biomass, due only to changes in the age structure of the populations. In the second scenario, hereafter referred to as "decreasing biomass," the spawning stock biomass decreases with increasing fishing pressure by allowing only a constant number of recruits. This scenario allows us to quantify the simultaneous effects of age structure change and changes in spawning stock biomass.

To investigate the effects that unequal egg survival can have on potential reproductive output, we calculated the number of potential recruits per populations by summing age-specific fecundities. We first assumed that all offspring have equal survival probabilities (hereafter referred to as the "fecundity method"). Second, we quantified potential recruitment with the survival of eggs being dependent on maternal factors such as size and age (hereafter referred to as the "viability method"). To investigate the effects of differing fishing pressure, under each of the stock–recruitment scenarios and for both recruitment production methods, we created sets of six different populations whose age-class structures are defined by the level of fishing pressure that the populations receive (Fig. 1).

Model species

To parameterise our model of a multiple spawner, we used life history traits from female Icelandic cod, as there have been recent investigations indicating that differences in the viability of eggs are related to female age, size, and condition (Marteinsdottir and Steinarsson 1998). Specifically, larval viability measured by the occurrence of swim bladders within the first 15 days from hatch has been related to egg and larval size as well as to larval growth rates and feeding efficiency (Marteinsdottir and Steinarsson 1998). Larger eggs are produced by older, larger females (Kjesbu 1989; Chambers and Waiwood 1996; Marteinsdottir and Steinarsson 1988). Therefore, we used mean length at age (centimetres) to calculate the corresponding mean egg diameter (millimetres) for that length at age (eqs. 1 and 2). We then calculated viability as a function of the frequency of swim bladder occurrence (Y) in Icelandic cod larvae and egg diameter (eqs. 3 and 4). For Icelandic cod, the mean weight, length, and fecundity at age were derived from spawning females on the main spawning ground south of Iceland in 1996 (n = 2917, G. Marteinsdottir et al., unpublished data; Marteinsdottir and Steinarsson 1998):

$$(1)\quad \text{Length}_{(\text{age})} = 43.4 + 6.52 \times \text{age};\ r^2 = 0.43$$

$$(2)\quad \text{Egg diameter}_{(\text{age})} = 1.23 + 0.002 \times \text{length}_{(\text{age})};\ r^2 = 0.31$$

$$(3)\quad \log(Y_{(\text{age})}/(1 - Y_{(\text{age})})) = -18.278 + 12.899 \times \text{egg diameter}_{(\text{age})};\ \chi^2 = 19.23,\ \text{df} = 43$$

$$(4)\quad \text{Viability}_{(\text{age})} = e^{Y_{(\text{age})}}/(1 + e^{Y_{(\text{age})}}).$$

To produce estimates of fecundity at age for all methods and scenarios, we calculated the numbers of eggs produced for each age-class based on the average weight at age (eqs. 5 and 6). To calculate population fecundity, the cumulative sum of the fecundities at age is produced by multiplying the number in each age-class by the age-specific fecundity:

$$(5)\quad \text{Weight}_{(\text{age})} = (198.3 \times (\text{age}^{1.837}))/1000;\ r^2 = 0.34$$

$$(6)\quad \text{Fecundity}_{(\text{age})} = -1\,396\,801 + 1\,044\,446 \times \text{weight}_{(\text{age})};\ r^2 = 0.93$$

Female Icelandic cod do not begin to mature until 4 years of age, with only 10–40% of the population reaching maturity in their fourth year. Not until cod reach 7–9 years of age are 100% of the individuals sexually mature (G. Marteinsdottir, unpublished data). In this study, we have defined each population as having 25% of 4-year-olds, 50% of 5-year-olds, and 75% of 6-year-olds spawning. For all other age-classes, 100% of the individuals are assumed to have spawned. We have not considered aspects of male life history traits in this study, as male cod do not seem to show any change in sperm quality with size (Trippel and Neilson 1992).As well, male cod may tend to mature slightly earlier than females, but the trend in more recent years has been to mature at an age similar to that of females (Jørgensen 1990; Trippel 1995).

Constant and decreasing biomass stock–recruitment scenarios

To create each set of six populations, we first calculate the numbers for each age-class, starting at the age of maturity:

$$(7)\quad \text{Number}_{(\text{age}+1,\text{population})} = \text{number}_{(\text{age},\text{population})} \times e^{-Z_{(\text{population})}}.$$

© 1999 NRC Canada

Fig. 1. Flow diagram demonstrating the pathways in which the production of potential recruits is calculated under the two main stock–recruitment scenarios, two types of recruit production, and the sets of six populations representing the six different levels of fishing pressure.

THE PRODUCTION OF POTENTIAL RECRUITS

Scenarios: Stock-Recruitment Relationships

Constant Biomass	*Decreasing Biomass*
Recruitment increases with increasing fishing pressure	Recruitment is constant with increasing fishing pressure

↓ ↓

Methods: Potential Recruit Production

Fecundity	*Viability*	*Fecundity*	*Viability*
Eggs have equal survival	Egg survival f(adult size)	Eggs have equal survival	Egg survival f(adult size)

↓ ↓ ↓ ↓

Populations: Fishing Pressure on 6 Populations

		Pop. 1	*Pop. 2*	*Pop. 3*	*Pop. 4*	*Pop. 5*	*Pop. 6*
		↓	↓	↓	↓	↓	↓
Natural Mortality	(M)	0.2	0.2	0.2	0.2	0.2	0.2
Fishing Mortality	(F)	0.0	0.2	0.4	0.6	0.8	1.0
Total Mortality	(Z)	0.2	0.4	0.6	0.8	1.0	1.2

The total instantaneous annual mortality (Z) for each of the six populations is made up of natural mortality (M = 0.2) and fishing mortality (F) (Fig. 1). Therefore, the six populations have different age-class structures (the numbers of individuals in each age-class) that result solely from the differences in total annual mortality. Within the six populations, each reproductive age-class (4–14) suffers the same constant annual mortality. Population 1 is the standard against which all quantitative comparisons are made, as it is considered to represent the "equilibrium" or "traditional" population, which experiences only natural mortality (M = 0.2). By design, in each set of populations, population 1 has the same number of individuals in each age-class and the same total spawning stock biomass.

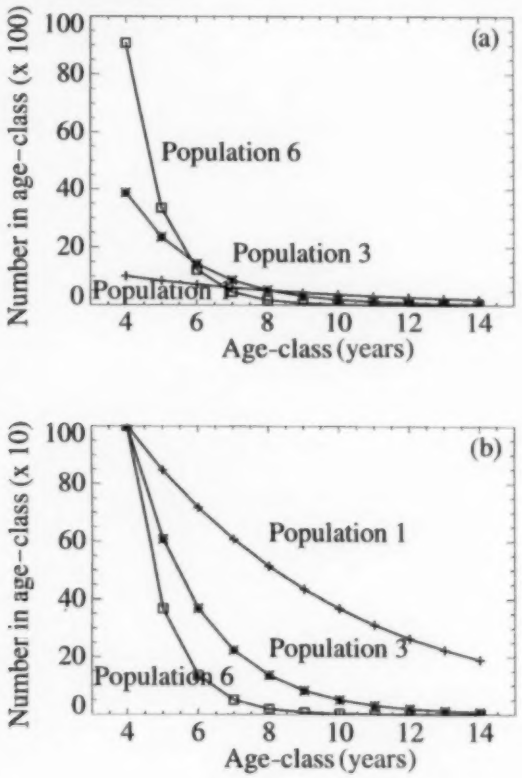
For the decreasing biomass stock–recruitment scenario, the numbers of recruits (at the age of sexual maturity) for each of the six populations is a constant (1000). This scenario represents one extreme of potential stock–recruitment relationships. When recruitment is held constant, then the effects of increasing fishing pressure continuously reduce the spawning stock biomass. Therefore, the subsequent numbers at each successively older age-class within each population are solely functions of the total annual instantaneous mortality (eq. 7). The spawning stock biomass (SSB) for each population is the sum of the numbers times mean weights at age of all the age-classes (eq. 8; Fig. 2). The proportions, by biomass, that each age-class represents are calculated using eq. 9. Under this stock–recruitment scenario, the populations with higher mortality rates (populations 4–6) have similar numbers of younger individuals but have greatly reduced biomass and are composed of far fewer older/larger fish:

$$(8)\quad SSB_{(population)} = \Sigma\,(number_{(age,\ population)} \times weight_{(age)})$$

$$(9)\quad Proportion_{(age,population)} = (number_{(age,population)} \times weight_{(age)})/SSB_{(population)}.$$

© 1999 NRC Canada

Fig. 2. Numbers in each age-class for populations 1, 3, and 6 under the (*a*) constant and (*b*) decreasing biomass stock–recruitment scenarios.



For the constant biomass scenario, the numbers at each age-class for each of the six populations were calculated using the same proportions (by biomass), per age-class, as were derived for the decreasing biomass scenario. The number of individuals within each age-class is calculated using eq. 10. Therefore, under this scenario, the populations with higher mortality rates (populations 4–6) have the same biomass as populations with less intensive fishing pressure (populations 1–3) but have much higher numbers of younger individuals and are composed of far fewer older/larger fish (Fig. 2). This scenario represents the other extreme of potential stock–recruitment relationships, that the effects of fishing mortality are completely compensated for and recruitment replaces all lost biomass:

$$(10)\quad \text{Number}_{(\text{age,population})} = (\text{proportion}_{(\text{age, population})} \times \text{SSB}_{(\text{constant})})/\text{weight}_{(\text{age})}.$$

The two stock–recruitment scenarios therefore allow us to quantify (*i*) the number of potential recruits produced due only to the effects of age structure change and (*ii*) the number of potential recruits produced due to the simultaneous effects of age structure change and changes in spawning stock biomass.

Fecundity and viability recruit production

In order to compare the numbers of potential recruits produced by each of the production methods (fecundity method: survival of all eggs is equal; viability method: survival of eggs depends on maternal factors), we must first calculate a "standard" survival rate for both methods. To calculate a standard survival rate, we assumed that population 1, with an annual instantaneous natural mortality of $Z = 0.20$, represents a stable state. Therefore, the standard fecundity survival rate of the eggs could be back-calculated by assuming that the total spawning stock fecundity of population 1, multiplied by a constant survival rate, must reproduce the total number of incoming recruits (1000). This standard fecundity survival rate (SFS = 2.51×10^{-8}) was then used in eq. 11 to calculate the number of potential recruits for the fecundity method for both sets of six populations under each stock–recruitment scenario:

$$(11)\quad \text{Potential recruits}_{(\text{fecundity})} = \Sigma(\text{number}_{(\text{age})} \times \text{fecundity}_{(\text{age})} \times \text{SFS}).$$

The viability method introduces a change in survival rates due to maternal age and size. Therefore, the standard viability survival rate must take on a slightly different value than the one that was calculated for the fecundity method for population 1 to also produce 1000 recruits. This standard viability survival rate (SVS = 4.36×10^{-8}) was then used in eq. 12 to calculate the total number of potential recruits for each population under the viability method for the two sets of six populations under both stock–recruitment scenarios:

$$(12)\quad \text{Potential recruits}_{(\text{viability})} = \Sigma(\text{number}_{(\text{age})} \times \text{fecundity}_{(\text{age})} \times \text{SVS} \times \text{viability}_{(\text{age})}).$$

Quantifying potential recruitment

We quantified differences in potential reproductive output between scenarios, production methods, and fishing pressures in two ways. First, we compared the number of potential recruits produced and, second, we compared the number per unit biomass of potential recruits produced with the expected number or expected number per unit biomass that the traditional method would predict. The expected number of potential recruits produced for each population (ENR) is calculated by multiplying the spawning stock biomass of each population by the standard number of potential recruits per unit biomass (eq. 13). The standard number of potential recruits per unit biomass (SRUB) is defined as the number of recruits produced by the standard population, population 1, divided by its biomass:

$$(13)\quad \text{ENR}_{(1-6)} = \text{SRUB} \times \text{SSB}_{(1-6)}.$$

The number of potential recruits per unit biomass (NRUB) for each population was calculated by dividing the number of recruits per population by its respective biomass:

$$(14)\quad \text{NRUB}_{(1-6)} = \text{recruits}_{(1-6)}/\text{SSB}_{(1-6)}.$$

One minus the NRUB/SRUB ratio produces what we have termed the percent overestimate of recruitment production. We call it an overestimate, as it represents the difference, per unit of spawning stock biomass, between the number of recruits that a traditional population (one with a full complement of age-classes) would be predicted to produce and the number of recruits that populations with increasing fishing pressure (i.e., truncated age-class structure) actually produce. The added effect of viability (over and above the effects of fecundity) is calculated by subtracting the percent overestimate produced by the fecundity method from the percent overestimate produced by the viability method.

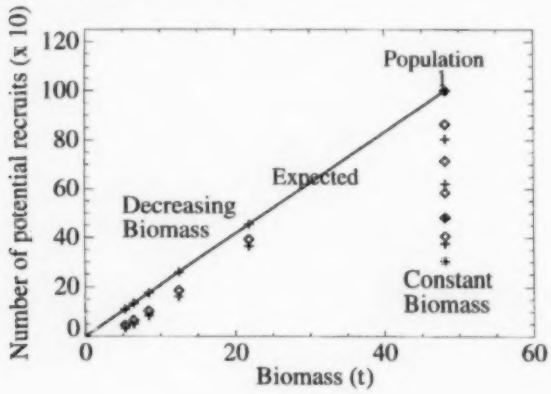
© 1999 NRC Canada

Table 1. Constant biomass scenario.

Population	Biomass (kg)	Expected number of recruits	Fecundity method number of recruits	Difference between expected and fecundity method	Viability method number of recruits	Difference between expected and viability method
1	48 140	1000	1000	0	1000	0
2	48 140	1000	864	136	805	195
3	48 140	1000	715	285	621	379
4	48 140	1000	584	416	478	522
5	48 140	1000	482	518	375	625
6	48 140	1000	405	595	305	696

Note: The numbers of expected potential recruits are compared with the actual number of recruits produced under the fecundity and viability methods of production. The difference between the expected number of recruits and those produced by the two methods of production is presented.

Fig. 3. Number of potential recruits produced versus the spawning stock biomass under the constant and decreasing biomass stock–recruitment scenarios. Population 1 is indicated on the figure, as it the standard reference point and has the same number of potential recruits under all scenarios and production methods. Numbers produced via the fecundity method are represented by diamonds; numbers produced via the viability method are represented by plus signs.



Results

Age-class structure under varying fishing pressure

The numbers at age for each of the sets of six populations of cod under the constant and decreasing biomass scenarios are presented in Fig. 2. The constant biomass scenario produces six populations that experience increasing levels of fishing pressure but retain a constant biomass due to the number of recruits being allowed to increase relative to decreases in spawning stock biomass. Under this scenario, the populations that experience heavier fishing pressure have greater numbers of cod. The increase is entirely due to the increase of numbers in the younger age-classes where the recruiting age-class (4) increases by a factor of 9 and age-class 5 increases by a factor of 4 between population 1 and population 6. The older age-classes, however, decrease in numbers, with age 10 showing a 94% reduction between population 1 and population 6. The decreasing biomass scenario produces six populations that experience increasing levels of fishing pressure but have constant recruitment. The subsequent populations have drastic declines in their spawning stock biomass and corresponding decreases in the numbers of fish in all age-classes. Under this scenario, cod numbers drop by 57% in age-class 5 and by 99% in age-class 10 between population 1 and population 6.

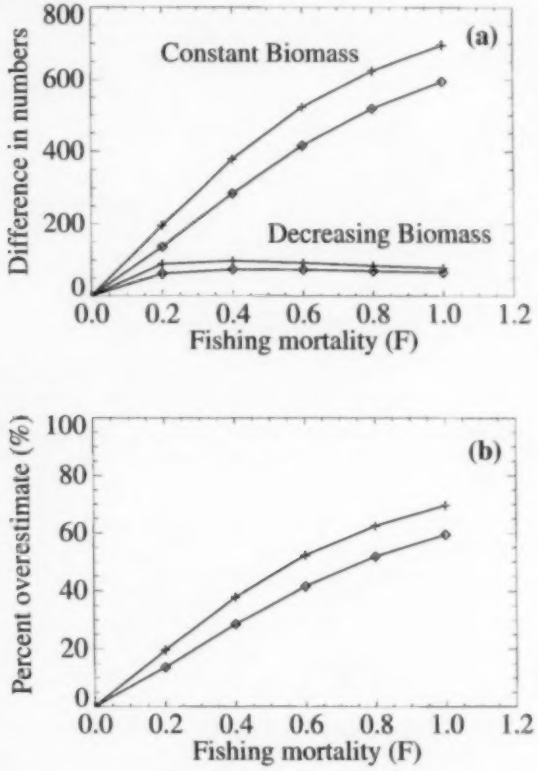
By design, the proportion by biomass of fish per age-class is the same under both the constant and the decreasing biomass stock–recruitment scenarios. Therefore, under both scenarios, within the populations that receive little or no fishing mortality (populations 1–3), the first three mature age-classes (ages 4–6) represent between 20 and 50% of the population spawning stock biomass. For the populations under moderate to high exploitation (populations 4–6), the first three mature age-classes represent the vast majority (70–85%) of the population spawning stock biomass. Spawning stock biomass is, of course, constant across all six populations under the constant biomass stock–recruitment scenario, but under the decreasing biomass scenario, relatively low levels of fishing pressure produce large differences in the spawning stock biomass of the different populations. In population 3, where $F = 0.4$, there a decrease of 75% in the spawning stock biomass compared with population 1. The spawning stock biomass of population 6, where $F = 1.0$, is 90% less than that of population 1.

Potential recruit production: numbers

Across stock–recruitment scenarios and recruit production methods, the populations that experience greater fishing pressure produce substantially fewer numbers of recruits. Under the constant biomass scenario, the expected number of recruits is always 1000. However, within those populations that experience greater fishing pressure, the age structure is severely truncated and the loss of those older/larger, more fecund individuals increases the difference between the expected number of recruits produced with the traditional assumptions and the actual number produced. Under the fecundity method of production (all eggs have equal survival), the number of potential recruits produced decreases from 1000 to 405 (Table 1; Fig. 3), which is a difference of 40.5% (Fig. 4*a*). The number of potential recruits produced via the viability method (eggs from larger/older females have increasing survival rates) shows an even greater decrease, with the extreme of only 30.5% of the expected 1000 recruits being produced in population 6 (Table 1; Figs. 3 and 4). Increasingly removing the more fecund, older/larger individuals causes a range of predicted numbers of potential recruits at a constant spawning stock biomass. This result suggests that age structure differences could be one of the main factors responsible for most stock–recruitment rela-

© 1999 NRC Canada

Fig. 4. (*a*) Difference between the expected and the produced absolute number of recruits for the constant and decreasing biomass biomass stock–recruitment scenarios for each of the six populations at increasing levels of fishing pressure and (*b*) percent overestimate of the difference between the expected and the produced number of recruits per unit of spawning stock biomass at increasing levels of fishing pressure. Numbers produced via the fecundity method are represented by diamonds; numbers produced via the viability method are represented by plus signs.



tionships having such large residual deviations at a given stock biomass (Fig. 3).

Under the decreasing biomass scenario, the age structure is truncated in a pattern similar to that under the constant biomass scenario. However, in this scenario, there is also a loss of spawning stock biomass within the populations that experience higher levels of fishing pressure. This loss of biomass decreases the number of expected potential recruits (Table 2). Therefore, even though the number of potential recruits produced via both the fecundity and viability methods showed dramatic decreases (as high as a 97% reduction in total recruit production; Fig. 3), the difference between the expected number and the actual number produced stays essentially constant, with an average difference of 69 for the fecundity method and 88 for the viability method, which equates to an error of 7–9% (Fig. 4*a*).

Potential recruit production: numbers per unit biomass

By design, when viewing the results in terms of per unit biomass, the constant and decreasing biomass scenarios collapse into the same output, as they contain the same proportion of biomass per age-class. The expected number of potential recruits per unit biomass is a constant (2.08×10^{-2}) for all populations and equals the number of recruits per unit biomass calculated for population 1. The produced number of potential recruits per unit biomass decreases across the populations with increasing fishing pressure (Table 3). Under the fecundity method, at the lowest levels of fishing pressure in population 2, where $F = 0.2$, the percent overestimation of potential recruits is 13.7%. As fishing pressure increases to 1.0 (populations 3–6), the overestimation of potential recruits, per population, continuously rises. However, the rate of increase of overestimation with increasing fishing pressure starts to decline between F values of 0.6 and 0.8, with a total overestimate of potential recruits of 59.5% within the most heavily fished population (Fig. 4*b*). Under the viability method of production, these percent overestimates increase from 19.5 to 69.6% as F increases from 0.2 to 1.0. Therefore, the added effect of differences in egg survival ranges from a 5.8 to a 10.7% overestimation (Table 3; Fig. 4*b*).

These results indicate that, when viewed on a per unit biomass basis, a decline in spawning stock biomass does not conceal the proportional changes in potential recruit production, as is the case when only the absolute number of recruits is analysed. This difference in results is due to the loss of older/larger individuals that are much more fecund, per unit of weight, than the younger fish left in the heavily fished populations. The number of eggs per gram of weight for a 4-year-old first-time spawner is 493 eggs/g, whereas an 8-year-old has 890 eggs/g. These results also suggest that for species whose first-time spawners have relatively low fecundities compared with older age-classes, the effects of even low levels of fishing pressure lead to large overestimates of potential recruits relative to the standard stock–recruitment relationships. However, as populations are more heavily fished and contain fewer and fewer older/larger individuals, the overestimate of potential recruits will reach a maximum.

Discussion

The use of spawning stock biomass as a measure of population fecundity has been criticised because it fails to account for changes in relative fecundity (number of eggs per weight of female) and egg survival that may result from differences in age, size, or condition (Hislop 1988; Kjesbu et al. 1991; Trippel 1998). This study, using a simple strategic model, quantifies how changes in the age composition of the spawning stock due to (*i*) increasing fishing pressures, (*ii*) different stock–recruitment relationships, and (*iii*) different potential recruit production methods could influence the reproductive output in terms of potential recruitment. The quantitative comparisons are made between a standard population where all age-classes experience only $M = 0.2$ and populations that suffer increasing levels of fishing pressure (F increases from 0.0 to 1.0). The results are viewed in terms of absolute numbers and numbers per unit biomass of potential recruits.

© 1999 NRC Canada

Table 2. Decreasing biomass scenario.

Population	Biomass (kg)	Expected number of recruits	Fecundity method number of recruits	Difference between expected and fecundity method	Viability method number of recruits	Difference between expected and viability method
1	48 140	1000	1000	0	1000	0
2	21 825	454	392	62	365	89
3	12 448	259	185	74	161	98
4	8 452	176	103	73	84	92
5	6 448	134	65	69	50	84
6	5 303	110	45	65	33	77

Note: The numbers of expected potential recruits are compared with the actual number of recruits produced under the fecundity and viability methods of production. The difference between the expected number of recruits and those produced by the two methods of production is presented.

Table 3. Potential recruits per unit biomass.

Population	Expected number of recruits	Fecundity method number of recruits	Fecundity method % overestimate	Viability method number of recruits	Viability method % overestimate	Additional effect of viability method
1	2.08×10^{-2}	2.08×10^{-2}	00.0	2.08×10^{-2}	00.0	00.0
2	2.08×10^{-2}	1.79×10^{-2}	13.7	1.67×10^{-2}	19.5	5.8
3	2.08×10^{-2}	1.49×10^{-2}	28.5	1.29×10^{-2}	37.5	9.4
4	2.08×10^{-2}	1.21×10^{-2}	41.6	0.99×10^{-2}	52.2	10.6
5	2.08×10^{-2}	1.00×10^{-2}	51.8	0.78×10^{-2}	62.5	10.7
6	2.08×10^{-2}	0.84×10^{-2}	59.5	0.63×10^{-2}	69.6	10.1

Note: The numbers of expected potential recruits produced per unit biomass (using the traditional assumption that the number of eggs per unit biomass is a constant) are compared with the actual number of potential recruits produced per unit biomass under the fecundity and viability methods of production. The percent overestimate of potential recruits that the traditional method would produce when compared with the two other methods of production is presented. The additional effect of the viability method (after the effect of the fecundity method is taken into account) is presented separately.

Age-class structure

The age-class structure of fish populations is largely determined by a combination of fishing pressure and stock–recruitment relationships. We found that even low to moderate levels of fishing pressure dramatically truncated the age-class structure of a population. An instantaneous fishing mortality value of 0.4 led to 53% of the population being made up of only the first three mature age-classes. An increase in F to 0.6 increased that proportion to 70%, with the first-time spawners (age-class 4) accounting for 30%. In Icelandic cod populations, as with most multiple-spawning species, the proportion of individuals within the first three maturing age-classes that actually spawn can be well below 100%, with the initial recruiting age-class having the lowest proportion of spawners. This suggests that even fish populations that receive low to moderate levels of fishing pressure have first-time spawners representing the vast majority of their spawning individuals. Percentages of first-time spawners caught in the commercial catch have been as high as 95% for Northeast Atlantic cod (Jørgensen 1990), and percentages of immature George's Bank cod,which are within the size range of being first-time spawners, have been as high as 42% of the commercial catch (Hunt 1996).

High levels of exploitation can also lead to lowering the age and size at which maturity is reached (Trippel 1995). This change in age at maturity can have a compensatory effect on egg production. Using a hypothetical stock of Atlantic cod, Trippel et al. (1997) demonstrated that lowering the age of maturity by 1 year led to a 16% increase in the number of eggs produced compared with what would have been produced given no decrease in the age of maturity. This clearly demonstrates that changes in life history characteristics need to be taken into account when calculating potential recruit production. We have not included these sorts of changes in our current strategic model, as the actual mechanism for changes in age and size at maturation is not the fishing pressure per se but may be due to the subsequent changes in population density (Trippel 1995). Also, environmental influences may be much more important in determining age and size at maturation (Kjesbu et al. 1991; Marshall et al. 1998), and including environmental variation is beyond the scope of this study.

Stock–recruitment relationships

Stock–recruitment relationships can take many different shapes due mainly to density-dependent and environmental factors. In this study, we consider two idealised extremes of stock–recruitment relationships: the constant and the decreasing biomass scenarios. The constant biomass scenario assumes that there is an increase in recruitment proportional to the loss of biomass such that all six populations maintain a constant spawning stock biomass. This scenario allows us to look at the effects on reproductive output due only to changes in age-class structure. The decreasing biomass scenario dictates a constant number of recruits for each population such that increased fishing pressure inevitably leads to a decrease in the population spawning stock biomass as well as changes in the structure of the age-classes. Reality, of course, is somewhere in between these two extremes, but by exploring the extremes, we hoped to show the largest possi-

© 1999 NRC Canada

ble quantitative range of effects and be able to identify the mechanisms responsible for the changes in reproductive output.

Potential recruit production: numbers

We found that even at a constant spawning stock biomass, our model predicts a wide range of potential numbers of recruits, decreasing with increasing fishing pressure (Fig. 3). The difference between the expected number of recruits predicted by the assumption within the traditional stock–recruitment relationship and the actual number produced can be as high as 60% in the heavily fished population. Once the potential maternal effects on egg survival are included in the model, the difference can be as high as 70% (Fig. 4*a*). This difference in the number of recruits, at the same spawning stock biomass, is driven by the decrease in the number of older/larger, more fecund individuals and not by changes in the stock biomass, as each population contains the equivalent number of younger individuals to maintain the biomass. This result suggests that age structure differences could be one of the main factors responsible for the large amount of residual deviance around most stock–recruitment relationships. And indeed, in the few studies so far where age structure has been taken into account, the stock–recruitment relationship was improved (Marshall et al. 1998; Martiensdottir and Thorarinsson 1998).

Under the decreasing biomass scenario, where age structure changes are simultaneous with changes in spawning stock biomass, we found that, relative to a traditional population, a heavily fished population suffers a loss of 95–97% of the number of potential recruits that it could produce (depending on whether the fecundity or viability method was used; Fig. 3). However, under this scenario, we did not find large differences between the expected and predicted number of potential recruits. In fact, we found only a small, almost constant error of 7–9% (Fig. 4*a*). This implies that when viewed in terms of expected numbers of recruits from a stock–recruitment relationship, the simultaneous decline in spawning stock biomass conceals the effect of the decrease in the number of older/larger individuals in the more heavily fished populations. The reason for the relatively small difference in expected and produced absolute numbers of recruits is that the declines in spawning stock biomass are so drastic. Even the lowest level of fishing pressure, where $F = 0.2$, leads to a 55% reduction in stock biomass relative to the traditional population. Therefore, the concealing of the effects of age structure changes by the simultaneous changes in spawning stock biomass is the most likely reason why the critical assumption in the traditional stock–recruitment relationship (that there is a constant mean number of eggs produced per unit biomass) has not been severely challenged.

Potential recruit production: numbers per unit biomass

However, if the results are viewed in terms of the number of potential recruits per unit of spawning stock biomass, then both stock–recruitment scenarios provide the same result. The percent overestimate of the expected number of recruits per unit biomass increases dramatically as fishing pressure increasingly removes the older/larger individuals, which are more fecund and more successful (higher egg viability) per unit of weight. Our model suggests that the percent overestimate of potential recruits very quickly rises from 14 to 60% as F increases from 0.2 to 1.0. When age- and size-related maternal effects on egg viability are considered, the overestimate of potential recruits can be another 5–10% higher. The relative quantity of this "error" suggests that this assumption needs to be addressed and that we cannot continue to produce stock–recruitment relationships that do not take into account potential changes in the number of eggs being produced per unit biomass due to changes in age structure.

Conclusions

This study provides further support for the developing paradigm that age and size structure of the spawning stock should be considered in fisheries management (Trippel et al. 1997; Mackenzie et al. 1998; Marshall et al. 1998; Marteinsdottir and Thorarinsson 1998; Trippel 1998). Our results imply that spawning stock biomass alone is a flawed index of recruitment due to the invalid assumption that egg number and quality stay constant per unit biomass. This view contrasts with the emphasis of current management advice on defining a threshold level of spawning stock biomass below which historic data suggest that the probability of poor recruitment increases. This view also sheds doubt on some of the developing ideas of fisheries management, such as the suggestion that total allowable catches and effort should only be adjusted enough to allow all fish to spawn at least once (Myers and Mertz 1998). This quantitative study suggests that allowing the management of the fishery to continue, as it is at present, such that the majority of spawning fish are first-time spawners, is a very inefficient use of the resource and a potentially risky option.

Acknowledgements

This work was partly supported by the European Union (contract FAIR-CT95-0084). We wish to thank the following colleagues and reviewers for constructive criticism of earlier drafts of this manuscript: Edward Trippel, Olav Kjesbu, Brian Mackenzie, Allejandro Gallegoa, and Mike Heath. Special thanks to X. Lambin for his helpful contributions.

References

Beverton, R.J.H., and Holt, S.J. 1957. On the dynamics of exploited fish populations. Fish. Invest. Ser. II. Mar. Fish. G.B. Minist. Agric. Fish. Food No. 19.

Chambers, R.C. 1997. Environmental influences on egg and propagule sizes in marine fishes. *In* Early life history and recruitment in fish populations. *Edited by* R.C. Chambers and E.A.Trippel. Fish and Fisheries Series 21. Chapman and Hall, London, U.K. pp. 63–102.

Chambers, R.C., and Waiwood, K.G. 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. **53**: 1986–2003.

Hislop, J.R.G. 1988. The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglifinus*, in British waters. J. Fish Biol. **32**: 923–930.

© 1999 NRC Canada

Hodder,V.M. 1965. The possible effects of temperature on the fecundity of Grand Bank haddock. Int. Comm. Northwest Atl. Fish. Spec. Publ. **6**: 515–522.

Hunt, J.J. 1996. Rates of sexual maturation of Atlantic cod in NAFO division 5Z6 and commercial fishery implications. J. Northwest Atl. Fish. Sci. **18**: 61–75.

Jørgensen, T. 1990. Long-term changes in age at sexual maturity of Northeast Arctic cod (*Gadus morhua* L.). J. Cons. Cons. Int. Explor. Mer, **46**: 235–248.

Kjesbu, O.S. 1989. The spawning activity of cod, *Gadus morhua* L. J. Fish Biol. **34**: 195–206.

Kjesbu, O.S., Klungsoyr, J., Kryvi, H., Witthames, P.R., and Greer Walker, M. 1991. Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. Can. J. Fish. Aquat. Sci. **48**: 2333–2343.

Knutsen, G.M., and Tilseth, S. 1985. Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. Trans. Am. Fish. Soc. **114**: 507–511.

Mackenzie, B., Tomkiewicz, J., Koster, F., and Nissling, A. 1998. Quantifying and desegregating the spawner effect: incorporating stock structure, spatial distribution and female influences into estimates of annual population egg production. ICES C.M. 1998/BB:11.

Marshall, C.T., Kjesbu, O.S., Yaragina, N.A., Solemdal, P., and Ulltang, Ø. 1998. Is spawner biomass a sensitive measure of the reproduction and recruitment potential of Northeast Arctic cod? Can. J. Fish. Aquat. Sci. **55**: 1766–1783.

Marteinsdottir, G., and Steinarsson, A. 1998. Maternal influence on the size and viability of Iceland cod (*Gadus morhua* L.) eggs and larvae. J. Fish Biol. **52**: 1241–1258.

Marteinsdottir, G., and Thorarinsson, K. 1998. Improving the stock–recruitment relationship in Icelandic cod (*Gadus morhua* L.) by including age diversity of spawners. Can. J. Fish. Aquat. Sci. **55**: 1372–1377.

Myers, R.A., and Mertz, G. 1998. The limits of exploitation: a precautionary approach. Ecol. Appl. **8**(Suppl. 1): S2165–S169.

Ricker, W.E. 1954. Stock and recruitment. J. Fish. Res. Board Can. **11**: 559–623.

Solemdal, P., Bergh, Ø., Finn, N., Fyhn, H.J., Grahl-Nielsen, O., Homme, O., Kjesbu, O.S., Kjørsvik, E., Opstad, I., and Skiftesvik, A.B. 1992. The effects of maternal status of Arcto-Norwegian cod on egg quality and vitality of early larvae. II. Preliminary results of the experiment in 1992. ICES C.M. 1992/G:79.

Solemdal, P., Kjesbu, O.S., and Fonn, M. 1995. Egg mortality in recruit- and repeat-spawning cod — an experimental study. ICES C.M. 1995/G:35.

Stearns, S.C., and Crandall, R.E. 1984. Plasticity for age and size at sexual maturity: a life history response to unavoidable stress. *In* Fish reproduction: strategies and tactics *Edited by* G.W. Potts and R.J. Wootton. Academic Press, London, U.K. pp. 13–33.

Trippel, E.A. 1995. Age at maturity as a stress indicator in fisheries. BioScience, **45**: 759–771.

Trippel, E.A. 1998. Egg size and viability and seasonal offspring production of young Atlantic cod. Trans. Am. Fish. Soc. **127**: 339–359.

Trippel, E.A., and Neilson, J.D. 1992. Fertility and sperm quality of virgin and repeat-spawning Atlantic cod (*Gadus morhua*) and associated hatching success. Can. J. Fish. Aquat. Sci. **49**: 2118–2127.

Trippel, E.A., Kjesbu, O.S., and Solemdal, P. 1997. Effects of adult age and size structure on reproductive output in marine fishes. *In* early life history and recruitment in fish populations. *Edited by* R.C. Chambers and E.A. Trippel. Fish and Fisheries Series 21. Chapman and Hall, London, U.K. pp 31–62.

© 1999 NRC Canada

Narrow size regime among individuals favors rapid growth in Arctic char (*Salvelinus alpinus*) juveniles

Teija Seppä, Nina Peuhkuri, Heikki Hirvonen, Anssi Laurila, Jorma Piironen, and Esa Ranta

Abstract: We studied whether social environment in the form of different body size regimes in rearing groups affects the growth of juvenile Arctic char (*Salvelinus alpinus*) in hatchery conditions. Young-of-the-year Arctic char were divided into groups of three different compositions (a wide, a bimodal, and a narrow size–frequency distribution) in which individuals would be subjected to different dominance and competitive relationships. After a 9-month growth period the fish in the narrow size distribution had grown the fastest in both length and weight. Also, total biomass gains were highest for fish originating from the narrow size distribution treatment. The adjusted mean biomasses for wide, bimodal, and narrow size regime treatments were 5400, 5000, and 5900 g, respectively. Our results give support to the hypothesis that individuals in size-sorted groups should show enhanced growth compared with individuals originating from a wider size–frequency distribution where more aggressive interactions are to be expected.

Résumé : Nous avons voulu établir si l'environnement social, c'est-à-dire dans le cas qui nous occupe diverses distributions de tailles corporelles dans des groupes d'élevage, influe sur la croissance des ombles chevaliers (*Salvelinus alpinus*) juvéniles en écloserie. On a divisé de jeunes ombles chevaliers de l'année en groupes caractérisés par trois compositions différentes (distributions de fréquence de tailles étendue, bimodale et étroite) dans lesquels les sujets se trouveraient soumis à des relations de dominance et de compétition différentes. Au terme d'une période de croissance de 9 mois, les poissons du groupe à distribution de tailles étroite ont montré la croissance la plus rapide tant en longueur qu'en poids. De plus, c'est dans ce même groupe qu'on a observé les gains de biomasse totale les plus élevés. Les biomasses moyennes ajustées dans les trois groupes étaient de 5 400 g (distribution de tailles étendue), 5 000 g (distribution bimodale) et 5 900 g (distribution étroite). Nos résultats corroborent l'hypothèse que les sujets se trouvant parmi des poissons de tailles semblables connaîtraient une croissance supérieure à celle de sujets se trouvant dans un groupe où la distribution de fréquence des tailles est plus étendue et où on doit s'attendre à observer davantage d'agressivité entre individus.

[Traduit par la Rédaction]

Introduction

Size is an important character of any living organism, as various fitness-related aspects of an individual's life often scale with body size (Persson et al. 1997). In fish, body size potentially changes throughout the entire life span of individuals owing to their indeterminate growth pattern (Wootton 1990; McDowall 1994). Individual growth, however, shows considerable plasticity, varying with a number of internal and external factors including the social environment that the fish experience (e.g., Jobling and Reinsnes 1986, 1987; Huntingford et al. 1993).

The effect of the social environment on body growth in fish is likely to be mediated mainly through competition for food resources (Metcalfe 1986), this being often asymmetric in accordance with the body size differences among individuals (e.g., Pitcher et al. 1986). Larger size may offer an advantage, especially in interference competition (Pianka 1974; Persson 1985), usually characterized by fighting and aggressive behavior (Milinski and Parker 1991). In salmonids the competitive relationships are commonly settled by clear dominance hierarchies where individuals high in dominance rank have better access to food resources (Adams and Huntingford 1996) and thereby grow better than the subordinate individuals (Metcalfe 1986; Metcalfe et al. 1992). The poorer growth of subordinate individuals may also result from socially induced stress (Ejike and Schreck 1980). Although body size does not seem to be the primary

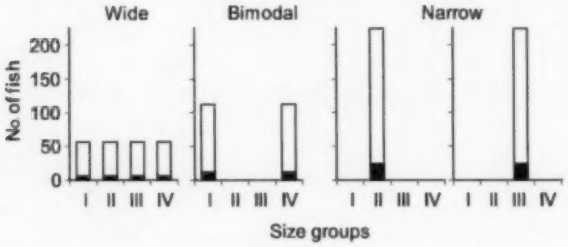
Received December 8, 1998. Accepted June 22, 1999.
J14930

T. Seppä,[1] **N. Peuhkuri, H. Hirvonen, A. Laurila, and E. Ranta.** Integrative Ecology Unit, Department of Ecology and Systematics, Division of Population Biology, University of Helsinki, P.O. Box 17, FIN-00014 Helsinki, Finland.
J. Piironen. Finnish Game and Fisheries Research Institute, Saimaa Fisheries Research and Aquaculture, Laasalantie 9, FIN-58175 Enonkoski, Finland.

[1]Author to whom all correspondence should be addressed. e-mail: teija.seppa@helsinki.fi

© 1999 NRC Canada

Fig. 1. Schematic presentation of the size groups of fish used in the different treatments: I, 6.6–8.5 cm; II, 8.6–10.5 cm; III, 10.6–12.5 cm; IV, 12.6–14.5 cm. The solid part of the bar represents the marked fish and the open part the unmarked fish.



factor determining an individual's rank in dominance hierarchy at the time of first feeding (Huntingford et al. 1990; Metcalfe et al. 1995), social status is yet often positively correlated with relative body size later on (Noakes 1980; Abbot et al. 1985; Metcalfe et al. 1992).

Some studies have shown an increased growth performance in size-sorted fish in experimental conditions (e.g., Gunnes 1976). In later studies, however, the positive effect of size grading on growth received very little support (Jobling and Reinsnes 1987; Wallace and Kolbeinshavn 1988; Baardvik and Jobling 1990).

In this work, we studied the patterns of fish growth in relation to the initial size distribution in juvenile Arctic char (*Salvelinus alpinus*). In Arctic char, larger individuals are usually dominant over their smaller conspecifics, and aggressive interactions are common even after dominance hierarchy has been established (Noakes 1980). We tested the hypothesis that in groups where individuals are of matching size, growth should be enhanced compared with groups with wider size distributions that allow the development of size-related dominance relationships potentially involving more aggression. Our emphasis was on both the average growth patterns and the growth patterns shown by individual fish, as the latter approach may increase our understanding of the growth of individual fish in different social environments.

Material and methods

Study subjects and rearing conditions

The study was carried out at Saimaa Fisheries Research and Aquaculture in eastern Finland. The study period lasted from October 29, 1996, to June 25, 1997. The fish used were hatchery-reared, young-of-the-year Arctic char that originated from the Kuolimo region in Lake Saimaa in eastern Finland. The fish were second generation in culture of wild-caught parents.

The fish were divided into four size-classes; small (6.6–8.5 cm), smaller medium sized (8.6–10.5 cm), larger medium sized (10.6–12.5 cm), and large (12.6–14.5 cm). They were reared in three kinds of social environments, i.e., treatments (Fig. 1). There were four replicate rearing tanks with 224 fish for each treatment. In the first treatment, there were 56 fish from each size-class (a wide size distribution), giving a total of 224 fish in each replicate. In the second treatment, half of the fish (112 individuals) were small and half of them were large (a bimodal size distribution). The third treatment consisted of 224 medium-sized fish per replicate (a narrow size distribution). Of these, two tanks consisted of smaller medium-sized fish and two of larger medium-sized fish. This is because our purpose was to create as narrow a size distribution as possible for each replicate. For logistic reasons, we could not use small or large fish in the narrow size regime. However, because it is the relative size difference between individuals that should be important in competitive and dominance relationships (Wankowski and Thorpe 1979; Dill 1983), our experimental procedure is not likely to affect the results emerging from the narrow distribution.

We followed both the length and weight increments of the fish throughout the study period by measuring them once a month. This protocol gave us a total of nine measurements for all fish. In addition to growth in length or weight as such, we also studied the length–weight relationships, as this subject has been mostly ignored in growth studies that mainly define growth as either weight (biomass) or length gain. However, our argument is that it is the length–weight relationship that reflects the condition of the individuals, therefore indicating how well they perform in a given environment.

In order to be able to follow the growth of the individual fish, we marked 24 fish in each tank divided equally between the size-classes. The marking was done with passive integrated transponder tags that were placed inside the body cavity of anaesthetized fish. Each tag (12 mm long, 2 mm in diameter) is uniquely coded and the code can be read by passing an antenna over the fish. All the fish were anaesthetized with MS 222 for the marking and also for the monthly measurements of weight and length.

The fish were reared in standard hatchery conditions in round rearing tanks with a diameter of 160 cm and water depth of 30 cm. In each tank, there was continuous water flow from a nearby lake at a rate of 18 L/min. The fish were fed ad libitum with automatic feeders continuously for 8 h/day. The amount of food was adjusted after every measurement according to the new biomasses in the tanks. Also, the size distribution of the fish in each tank was taken into consideration when adjusting the feeding by providing the appropriate particle size of feed. The photoperiods were 8 h light : 16 h dark from the beginning of the experiment until April 30 (winter) and 13 h light : 11 dark from May onwards (summer), both with 10-min dimming periods. The water temperatures ranged between +1 and +16°C during the study following ambient water temperature in the lake. None of the experimental tanks suffered from diseases and the mortality in the experiment was very low, less than 10%. The initial fish biomasses in the tanks ranged from 1380 to 2760 g and the biomasses were allowed to change throughout the experiment.

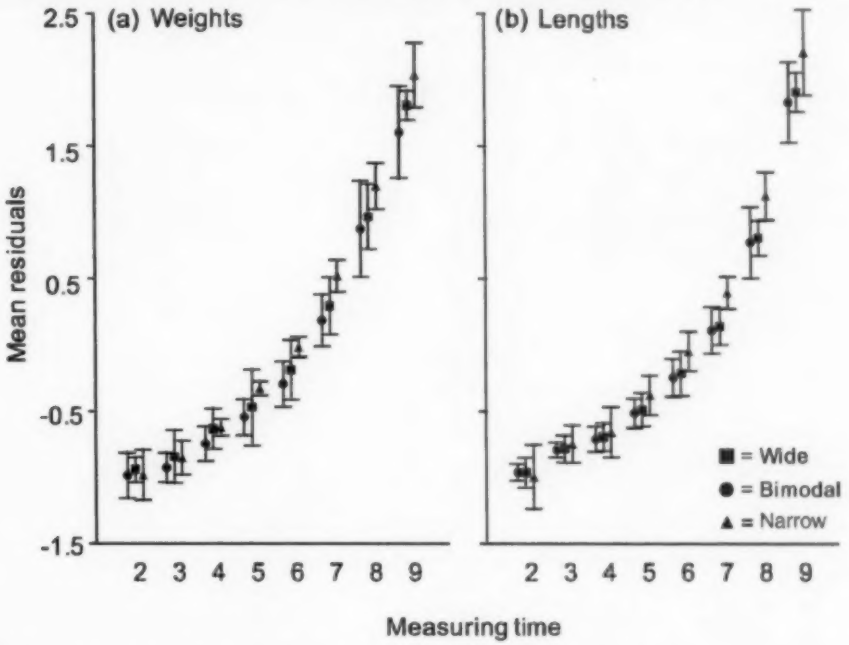
Statistical analyses

To test for differences in individual growth with the marked fish among the three treatments, we first used a transformation procedure where the size (log-transformed length or weight) of each marked fish at each subsequent time point from the second measuring time onwards was regressed on the size of that fish at the beginning of the experiment. We used the residuals from this model in order to minimize the effects of the initial sizes of individuals on their growth (Wootton 1990). Using the averages of the residuals for each tank, we tested whether social environment affects the growth of the fish in length and (or) weight. Since the observations at different time points are not independent, we used univariate repeated measures analysis of variance (ANOVA) in the analyses (Zar 1996). This also allowed us to assess whether the growth in the course of time differed among the treatments.

We also calculated the average residuals from the model for each size group in each tank at each measuring time to test for differences in growth within the size group in different treatments. This was done by comparing the growth in each size group separately with univariate repeated measures ANOVA. For the purpose of this analysis, both the smaller medium-sized groups and the larger medium-sized groups were pooled under medium-sized fish to give an even number of replicates for the treatments.

© 1999 NRC Canada

Fig. 2. Average (*a*) weight (*b*) and length residuals of the fish in different size regime treatments during nine measuring times. The values are regressed on the weights and lengths at the beginning of the growth period. Error bars represent the 95% confidence limits.



Measuring the length–weight relationship does not call for individually marked fish, so data from all the fish were used in the following analysis. To eliminate the effect of fish size on the relationship, we first fitted a linear regression model to the data by regressing log-transformed weights on log-transformed lengths at different time points. We used the tank-specific means of the residuals at the nine different measuring times to test with univariate repeated measures ANOVA whether the length–weight relationships among different treatments differ as experimental time proceeds. We chose residuals instead of the traditional condition factor in order to assess the condition (or energy allocation between weight and length) of individuals in different treatments, as the former method has been shown to control for the confounding effect of body size (Reist 1985; Jacob et al. 1996).

We also calculated the coefficients of variation (CV) for weights and lengths in each tank at each time step in order to investigate whether and how the CVs of size in different treatments changed with time. These were also tested with univariate repeated measures ANOVA.

Finally, we also tested with all the fish whether there were differences in relative biomass gain among different treatments by using an analysis of covariance (ANCOVA), where the total biomass of each tank at the beginning of the experiment was used as a covariate to control for the initial differences in the biomasses.

All statistical tests were done by using the SYSTAT6 statistical package (Wilkinson 1996).

Results

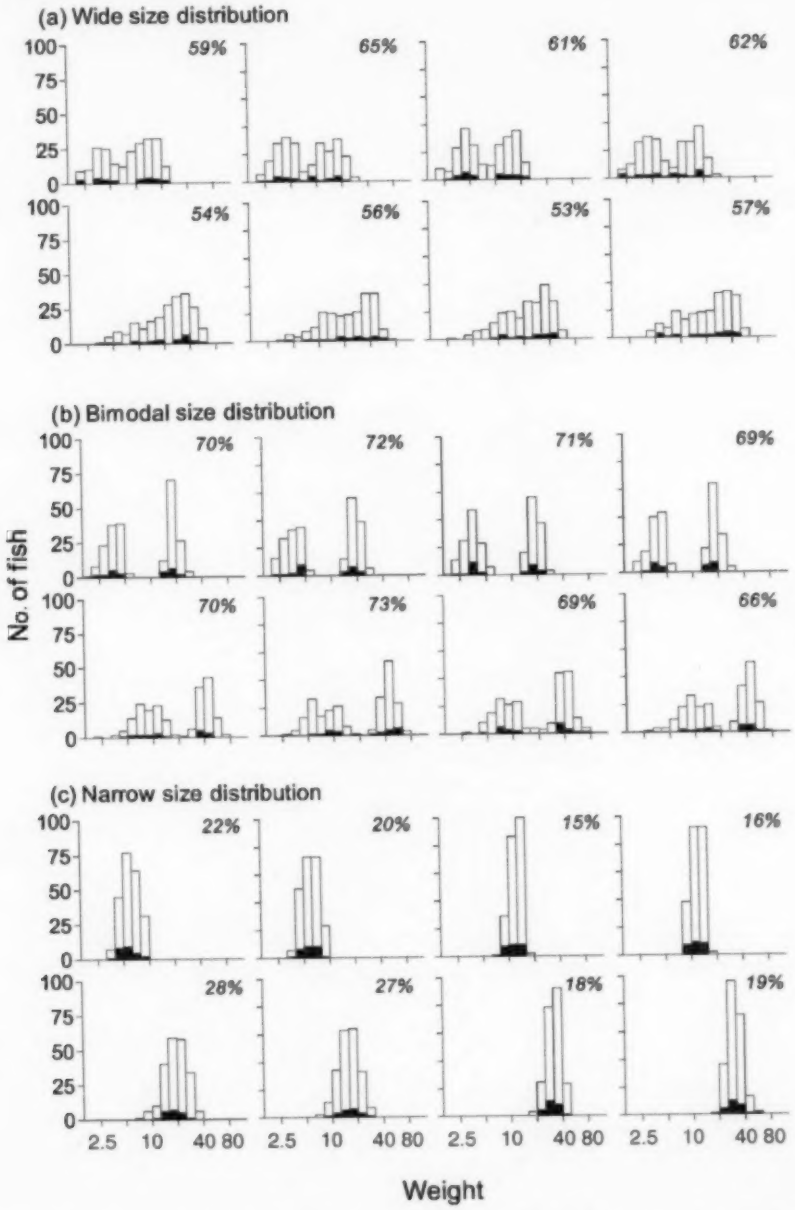
The individually marked fish in the narrow size distribution groups grew on average better than those in the groups with a wider size distribution. This could be seen in the significant treatment effect of the means of the residuals for weight (repeated measures ANOVA, $F_{2,9} = 5.85$, $p = 0.024$). Time had also an impact on the weight gain ($F_{7,63} = 1646.62$, $p < 0.001$), and there was a significant interaction between time and treatment ($F_{14,63} = 3.43$, $p < 0.001$), indicating that the weight increment in the course of time differed among the three size distributions so that at the end of the experiment, the weight gain was largest in the fish from the narrow size regime (Fig. 2*a*). Length data showed similar results, with significant treatment, time, and time and treatment interaction effects ($F_{2,9} = 6.58$, $p = 0.017$; $F_{7,63} = 1779.21$, $p < 0.001$; and $F_{14,63} = 4.05$, $p < 0.001$, respectively) (Fig. 2*b*).

The higher growth rate in fish grown in the narrow size distribution was also found when we tested for differences in growth between initially similar-sized fish in different treatments. Medium-sized fish grew better when kept with similar-sized fish than when they were a part of a group of fish with a wider size distribution (repeated measures ANOVA, time × treatment $F_{7,42} = 2.80$, $p = 0.02$). In lengths the results were similar, with the time and treatment interaction showing a tendency towards better growth in fish from the narrow size distribution ($F_{7,42} = 2.09$, $p = 0.07$). In groups of small and large fish, no significant differences in growth in length or weight between treatments were found (small fish: length, treatment $F_{1,6} = 0.03$, time × treatment $F_{7,42} = 0.22$; weight, treatment $F_{1,6} = 1.24$, time × treatment $F_{7,42} = 0.16$; large fish: length, treatment $F_{1,6} = 0.06$, time × treatment $F_{7,42} = 0.18$; weight, treatment $F_{1,6} < 0.01$, time × treatment $F_{7,42} = 0.71$).

With all the fish, no significant differences were found in the length–weight relationships among different treatments ($F_{2,9} = 0.20$, $p = 0.822$). Also, neither time ($F_{8,72} = 0.001$, $p = 1.000$) nor time and treatment interaction significantly

© 1999 NRC Canada

Fig. 3. Frequency distributions of fish weight in four replicate tanks in each treatment at the beginning and end of the growth period. In each treatment, the upper panels represent the situation at the beginning of the experiment and the lower panels that at the end of the experiment. The solid parts of the bars represent the marked fish and the open parts the unmarked fish. CVs are given at the top of each panel. Note the log scale on the *x*-axis.



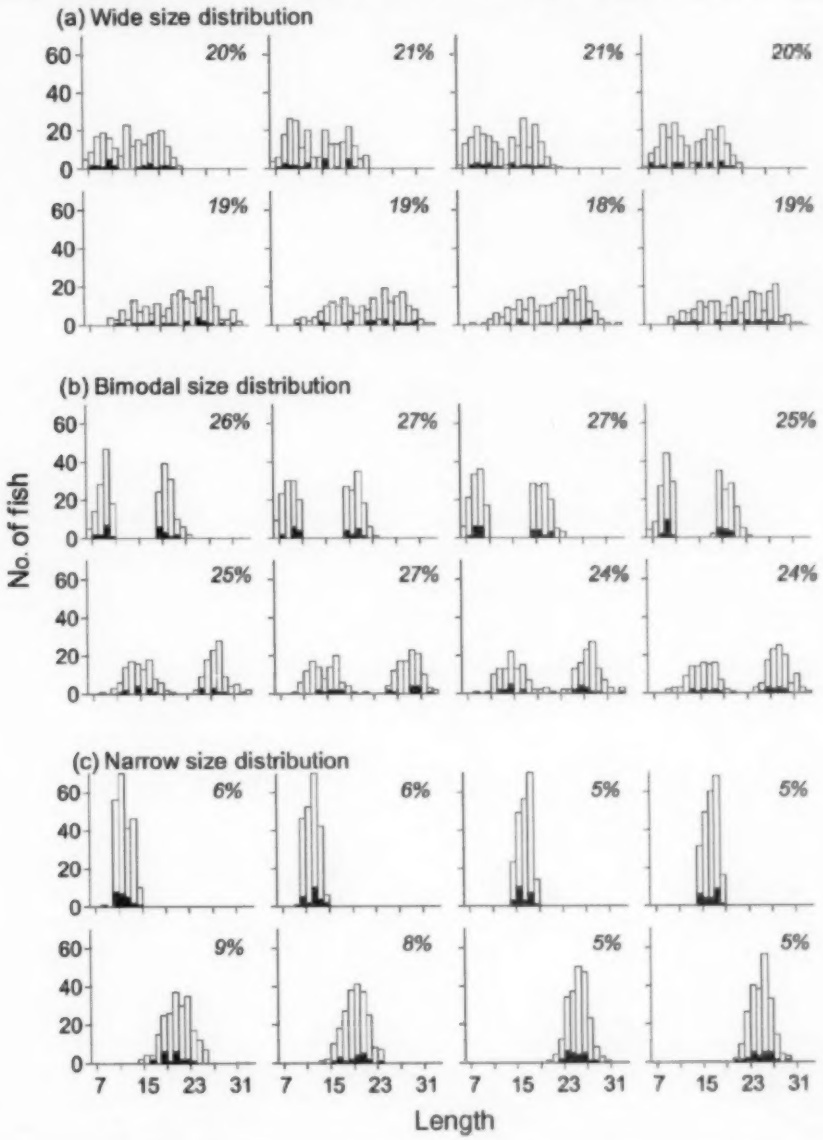
affected the length–weight relationship of the fish ($F_{16,72}$ = 1.248, p = 0.255).

There was a significant treatment effect on the CVs of weight in the experimental groups ($F_{2,9}$ = 298.23, p < 0.001).This was, however, expected, as the CVs already differed at the beginning of the experiment due to the experimental setup. The CVs also changed with time ($F_{8,72}$ = 49.79, p < 0.001), and, most importantly, there was a significant interaction between time and the size distribution ($F_{16,72}$ = 17.11, p < 0.001). This suggests that the pattern of change in the CV of weight differed among the three treatments in relation to time. The CVs in both the wide and bimodal size distributions became smaller and the CVs in the narrow size distribution became larger (Fig. 3).

The CVs for length showed results similar to those of weight, with the size distribution effect ($F_{2,9}$ = 472.69, p <

© 1999 NRC Canada

Fig. 4. Frequency distributions of fish length in four replicate tanks at the beginning and end of the growth period. In each treatment, the upper panels represent the situation at the beginning of the experiment and the lower panels that at the end of the experiment. The solid parts of the bars represent the marked fish and the open parts the unmarked fish. CVs are given at the top of each panel.



0.001) and time and treatment interaction ($F_{16,72}$ = 13.42, p < 0.001) being statistically significant (Fig. 4).

The homogeneity of slopes assumption for ANCOVA was fulfilled (ANCOVA, $F_{2,6}$ = 0.04, p = 0.961), i.e., there is no interaction between the dependent variable and the covariate. Both the size distribution ($F_{2,8}$ = 8.86, p = 0.009) and the initial body size ($F_{1,8}$ = 134.76, p < 0.001) had a statistically significant effect on the relative biomass gain among the different size regimes (Table 1). Pairwise comparisons with linear contrasts showed that the fish in the narrow size distribution had grown better in terms of relative biomass gain than fish in either the wide or bimodal size distribution ($F_{1,8}$ = 17.54, p = 0.003 and $F_{1,8}$ = 6.45, p = 0.035, respectively). Comparison between the wide and bimodal size distributions showed a tendency towards a higher relative biomass gain in the wide size distribution ($F_{1,8}$ = 3.96, p = 0.082).

Discussion

Although many studies have failed to show any advantage of size-sorting Arctic char (Jobling and Reisnes 1987; Wallace and Kolbeinshavn 1988) in an aquacultural setting or have even shown a growth disadvantage (Baardvik and Job-

© 1999 NRC Canada

Table 1. Adjusted least squared means and SEs given by ANCOVA for biomasses in different size regime treatments.

Size regime	*N*	Adjusted least squares mean	SE
Wide	4	5392.9	133.2
Bimodal	4	5002.0	142.0
Narrow	4	5879.2	139.6

ling 1990), we found a higher growth in both length and weight in Arctic char reared in a narrow size distribution compared with the groups with wider size distributions. The same patterns of growth were found when we compared individually marked fish of similar sizes in different treatments. This confirms that the higher growth rates observed in the narrow size regime fish are independent of fish size. Gunnes (1976) found a faster growth rate in Atlantic salmon (*Salmo salar*) that were size graded at 6 months old compared with groups that were size graded a few months older. He argued that size sorting would be advantageous only if size sorting young fish, before the establishment of size hierarchies. At the start of our experiment the fish were 8 months of age, but unlike in Gunnes' (1976) study, rearing fish in a narrow size regime at this age still had an positive effect on growth.

The growth of fish has received a lot of attention in fishery and aquaculture science because it can be translated directly into profit to the aquaculture industry. This type of approach often ignores the different decisions that individuals make in reaction to their environment (Dill 1983, 1987; Lima and Dill 1990). Our result of higher growth at the individual level in narrow size regime groups can also be seen in the relative biomass gain in the rearing tanks. This is a contradictory result to those of Jobling and Reisnes (1987), Wallace and Kolbeinshavn (1988), and Baardvik and Jobling (1990). These differences in results of the effects of size sorting may be due to differences in experimental setup. The other studies were done by dividing the population into two halves and testing for differences between "smaller than average," "larger than average," and "unsorted" fish. In our experiment the narrow size distribution group was strictly defined with only 2 cm difference in length between the largest and smallest fish within the groups. Braaten and Boge (1983) reported a higher growth rate in sorted fish groups compared with unsorted, but their work was done strictly for practical aquaculture purposes and lacked replication; therefore, it was not possible to test the results statistically.

Adams and Huntingford (1996) found in pairwise competition tests with Arctic char that when food supply was limited, the most aggressive individuals had better access to food resources. However, when the same individuals were transformed to rearing conditions with higher fish densities and with less restricted food levels, individuals' earlier competitive history did not correlate with their subsequent performance. It was suggested that different behavioral traits could be successful in different competitive environments. In our experiment the fish were fed in excess and the individuals were presumably not able to monopolize the food resources because the water drift spread the food particles quickly away from the feeder. In spite of this, interference competition might still lead to a reduction in food intake rate of some individuals because of the aggressive behavior involved (Milinski and Parker 1991). However, when fish are of similar size, the fighting costs involved in attacking another individual may be too high (Maynard Smith and Parker 1976), and as a result the fish in a narrow size distribution group may avoid showing aggressive behavior. Therefore, all the fish in tanks with little variation in individual body sizes are able to gain access to a share of the food. Low levels of aggression and the resulting high levels of growth are often coupled with low levels of variation in the growth rates (Jobling and Baardvik 1994). In our experiment the CVs of the weights and lengths became larger with time in the treatment with highest overall growth rate. The reason for this contradictory result could be in the experimental design. Our narrow size distribution was extremely narrow and the growth rates of individual fish were not homogeneous enough to keep them inside the limits of just a few centimetres, resulting in a higher CV in groups with high growth rates.

Aggression may be reduced in the tanks with a narrow size–frequency distribution of fish also because of the confusion that a school of similar-sized fish may create. Keenleyside and Yamamoto (1962) showed that when crowded, most juvenile Atlantic salmon in a group start showing schooling behavior, while a few individuals stayed as territory holders. If an individual strays from the school and is attacked by a territory holder, it can quickly escape the attacks by disappearing back into the group, thus reducing the time and effect of aggression. This may be especially effective when all the fishes in the school are of the same size, therefore reducing aggressive encounters in the groups of similar-sized fish, which in turn may result in the observed higher growth rates in the fish in the narrow size distribution.

It has been shown in juvenile coho salmon (*Oncorhynchus kisutch*) that length, rather than weight, is the most important predictor of dominance status in this species (Holtby et al. 1993). Depending on the correct indicator of size, this might result in the allocation of energy available differently into growth in length or growth in weight. Fish in a narrow size regime are competing with each other to gain a dominant position in the fish group by allocating energy into the character that is the most important predictor of the dominance status. We did not find clear differences in the length–weight relationships among different treatments when tested with all the fish available due to the low sample sizes. We suggest that in future studies, it would be advantageous to take a closer look at both weight and length and the relationships between them when studying growth patterns in fish.

We have demonstrated that the size distribution of fish has an impact on the growth of individuals. Individual fish growing in a narrow size range show higher growth rates in both weight and length than fish originating from a wider size–frequency distribution. This can be seen also when comparing individual similar-sized fish in different treatments. Also, the relative biomass gain is higher in the groups of fish reared in a narrow size range. A possible explanation for these findings could be that the threshold for attacking similar-sized individuals is so high that individuals

© 1999 NRC Canada

avoid aggression in narrow size distribution groups and thereby grow better than individuals in groups with a wider size–frequency distribution.

Acknowledgements

We are indebted to Hannu Rita for his insightful advice on the statistical treatment of the data. The Finnish Game and Fisheries Institute, Saimaa Fisheries Research and Aquaculture, provided the fish and good working facilities. Katriina Lahti, Susanna Pakkasmaa, and many others helped us with the laborious monthly measurements of the fish. This study was funded by the Academy of Finland, University of Helsinki, and Finnish Game and Fisheries Institute.

References

Abbot, J.C., Dunbrack, R.L., and Orr, C.D. 1985. The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). Behaviour, **92**: 241–253.

Adams, C.E., and Huntingford, F.A. 1996. What is a successful fish? Determinants of competitive success in Arctic char (*Salvelinus alpinus*) in different social contexts. Can. J. Fish. Aquat. Sci. **53**: 2446–2450.

Baardvik, B.M., and Jobling, M. 1990. Effect of size-sorting on biomass gain and individual growth rates in Arctic charr, *Salvelinus alpinus* L. Aquaculture, **90**: 11–16.

Braaten, B., and Boge, E. 1983. Sortering av smolt — praktiske konsekvenser for oppdrettere. Nor. Fiskeoppdrett, **8**: 16–17. (In Norwegian.)

Dill, L.M. 1983. Adaptive flexibility in the foraging behaviour of fishes. Can. J. Fish. Aquat. Sci. **40**: 398–408.

Dill, L.M. 1987. Animal decision making and its ecological consequences: the future of aquatic ecology and behaviour. Can. J. Zool. **65**: 803–811.

Ejike, C., and Schreck, C.B. 1980. Stress and social hierarchy rank in coho salmon. Trans. Am. Fish. Soc. **109**: 423–426.

Gunnes, K. 1976. Effect of size grading young Atlantic salmon (*Salmo salar*) on subsequent growth. Aquaculture, **9**: 381–386.

Holtby, L.B., Swain, D.P., and Allan, G.M. 1993. Mirror-elicited agonistic behaviour and body morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **50**: 676–684.

Huntingford, F.A., Metcalfe, N.B., Thorpe, J.E., Graham, W.D., and Adams, C.E. 1990. Social dominance and body size in Atlantic salmon parr, *Salmo salar* L. J. Fish Biol. **36**: 877–881.

Huntingford, F.A., Metcalfe, N.B., and Thorpe, J.E. 1993. Social status and feeding in Atlantic salmon *Salmo salar* parr: the effect of visual exposure to a dominant. Ethology, **94**: 201–206.

Jacob, M.E., Marshall, S.D., and Uetz, G.W. 1996. Estimating fitness: a comparison of body condition indices. Oikos, **77**: 61–67.

Jobling, M., and Baardvik, B.M. 1994. The influence of environmental manipulations on inter- and intra-individual variation in food acquisition and growth performance of Arctic charr, *Salvelinus alpinus*. J. Fish Biol. **44**: 1069–1087.

Jobling, M., and Reinsnes, T.G. 1986. Physiological and social constraints on growth of Arctic charr, *Salvelinus alpinus* L.: an investigation of factors leading to stunting. J. Fish Biol. **28**: 379–384.

Jobling, M., and Reinsnes, T.G. 1987. Effect of sorting on size–frequency distributions and growth of Arctic charr, *Salvelinus alpinus* L. Aquaculture, **60**: 27–31.

Keenleyside, M.H.A., and Yamamoto, F.T. 1962. Territorial behaviour of juvenile Atlantic salmon (*Salmo salar* L.). Behaviour, **19**: 139–169.

Lima, S.L., and Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospects. Can. J. Zool. **68**: 619–640.

Maynard Smith, J., and Parker, G.A. 1976. The logic of asymmetric contests. Anim. Behav. **24**: 159–175.

McDowall, R.M. 1994. On size and growth in freshwater fish. Ecol. Freshwater Fish, **3**: 67–79.

Metcalfe, N.B. 1986. Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. J. Fish Biol. **28**: 525–531.

Metcalfe, N.B., Wright, P.J., and Thorpe, J.E. 1992. Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic salmon (*Salmo salar*). J. Anim. Ecol. **61**: 585–589.

Metcalfe, N.B., Taylor, A.C., and Thorpe, J.E. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim. Behav. **49**: 431–436.

Milinski, M., and Parker, G.A. 1991. Competition for resources. *In* Behavioural ecology. An evolutionary approach. 3rd ed. *Edited by* J.R. Krebs and N.B. Davies. Blackwell Scientific Publications Ltd., Oxford, U.K. pp. 137–168.

Noakes, D.L.G. 1980. Social behavior in young charrs. *In* Charrs: salmonid fishes of the genus *Salvelinus*. *Edited by* E.K. Balon. Dr. W. Junk, The Hague, The Netherlands. pp. 683–701.

Persson, L. 1985. Asymmetrical competition: are larger animals competitively superior? Am. Nat. **126**: 261–266.

Persson, L., Diehl, S., Eklöv, P., and Christensen, B. 1997. Flexibility in fish behaviour: consequences at the population and community levels. *In* Behavioural ecology of teleost fishes. *Edited by* J.G. Godin. Oxford University Press, Oxford, U.K. pp. 316–343.

Pianka, E.R. 1974. Niche overlap and diffuse competition. Proc. Natl. Acad. Sci. U.S.A. **71**: 2141–2145.

Pitcher, T.J., Magurran, A.E., and Allan, J.R. 1986. Size-segregative behaviour in minnow shoals. J. Fish Biol. **29**(Suppl. A): 83–95.

Reist, J.D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Can. J. Zool. **63**:1429–1439.

Wallace, J.C., and Kolbeinshavn, A.G. 1988. The effect of size grading on subsequent growth in fingerling Arctic charr, *Salvelinus alpinus* (L.). Aquaculture, **73**: 97–100.

Wankowski, J.W., and Thorpe, J.E. 1979. Spatial distribution and feeding in Atlantic salmon, *Salmo salar* L., juveniles. J. Fish Biol. **14**: 239–247.

Wilkinson, L. 1996. SYSTAT for Windows: statistics. SYSTAT, Chicago, Ill.

Wootton, R.J. 1990. Ecology of teleost fishes. Chapman and Hall, London, U.K.

Zar, J.H. 1996. Biostatistical analysis. 3rd ed. Prentice-Hall, Englewood Cliffs, N.J.

© 1999 NRC Canada

Elemental distribution within polymorphic inconnu (*Stenodus leucichthys*) otoliths is affected by crystal structure

Randy Brown and Kenneth P. Severin

Abstract: The chemistry and crystal structure of sagittal otoliths from inconnu (*Stenodus leucichthys*) were examined optically, with an electron microprobe, a scanning electron microscope, and with X-ray diffraction techniques. The distributions of strontium (Sr), sodium (Na), potassium (K), and calcium (Ca) were determined with line scans and area maps of thin, transverse otolith sections. Regions depleted in Sr, Na, and K were found to be discordant with optical annuli and were optically distinct from other regions of the otoliths. These patterns of trace element depletion cannot be explained by models of otolith growth that are based on ionic composition of endolymph fluids as the sole control of otolith composition. Electron micrographs showed the depleted regions of the otoliths to be of a different crystal structure than other regions of the otoliths. X-ray diffraction analyses revealed the presence of vaterite in otoliths with depleted regions, while otoliths without depleted regions showed no evidence of vaterite. The depleted areas may be composed of vaterite, and the crystal structure of vaterite may prevent certain trace elements from incorporating in the otolith. Scientists using fish otolith chemistry to infer environmental conditions or life history should be aware that elemental abundance within otoliths may be affected by other processes as well.

Résumé : On a examiné optiquement la chimie et la structure cristalline des otolithes sagittaux de l'inconnu (*Stenodus leucichthys)* avec une microsonde électronique, un microscope à balayage électronique et des techniques de diffraction de rayons X. On a déterminé les distributions du strontium (Sr), du sodium (Na), du potassium (K) et du calcium (Ca) par balayage linéaire et cartographie des surfaces de minces coupes transversales des otolithes. Il y avait discordance entre les anneaux optiques et les régions pauvres en Sr, en Na et en K, et ces dernières étaient optiquement distinctes des autres régions des otolithes. Ces profils de déplétion des éléments traces ne peuvent être expliqués par les modèles de croissance des otolithes qui, pour caractériser la composition des otolithes, ne prennent en considération que la composition ionique des fluides endolymphatiques. Les micrographes électroniques ont montré que les régions appauvries des otolithes ont une structure cristalline différente de celle des autres régions. Les analyses par diffraction de rayons X ont révélé la présence de vatérite, et la structure cristalline de celle-ci pourrait empêcher certains éléments traces de s'incorporer aux otolithes. Les scientifiques qui utilisent la chimie des otolithes des poissons comme indices des conditions environnementales ou des cycles vitaux devraient avoir à l'esprit que l'abondance des divers éléments dans les otolithes peut aussi être liée à d'autres processus.

[Traduit par la Rédaction]

Introduction

Otoliths are mineral structures that lie within endolymphatic hollows associated with the semicircular canal network near the brain in teleost fish (Platt and Popper 1981). They function in hearing and balance. Otoliths are composed primarily of calcium carbonate amid a proteinaceous matrix (Degens et al. 1969). They grow throughout a fish's life as material precipitates on the outer surface. In many species, growth increments, similar to tree rings, are apparent both on daily intervals for juvenile fish (Campana and Neilson 1985) and on annual intervals for adults (McFarlane and Beamish 1995). Otoliths attain various shapes that are species specific and tend to be similar for related species (Morrow 1979; Lowenstam and Weiner 1989). Otoliths are generally considered to be insoluble in situ, containing an uninterrupted record of life history information within their growth increments (Gauldie and Nelson 1990). However, Pannella (1980) cautioned that otoliths must be viewed as potential contributors to whole-body calcium (Ca) metabolism, particularly during times of extreme stress (Mugiya and Uchimura 1989).

In recent years, fisheries scientists have used elemental information associated with growth increments from otoliths to describe life history events and patterns of movement of many fishes. Most of these studies based their interpretations on the assumption that the environmental conditions that a fish experiences (salinity, temperature, seawater chemistry) are major determinants of the chemical composition of otoliths (Secor 1992; Babaluk et al. 1997; Tzeng et al. 1997). Laboratory experiments suggest that this is a valid assumption (Mugiya and Tanaka 1995; Secor et al. 1995; Farrell

Received February 17, 1999. Accepted June 22, 1999.
J15022

R. Brown.[1] U.S. Fish and Wildlife Service, Fishery Resource Office, 101 12th Ave., Fairbanks, AK 99701, U.S.A.
K.P. Severin. Department of Geology and Geophysics, University of Alaska Fairbanks, Box 755780, Fairbanks, AK 99775-5780, U.S.A.

[1]Author to whom all correspondence should be addressed.
e-mail: randy_j_brown@fws.gov

© 1999 NRC Canada

and Campana 1996). Other studies indicate that physiological and other unknown factors may also play important roles in the chemical composition of otoliths, complicating simple interpretations of microchemical data (Kalish 1991; Sadovy and Severin 1994; Tzeng 1996). An improved understanding of the mechanisms that control otolith growth and chemical composition will allow more accurate interpretations of the distribution of elements in otoliths.

Otoliths are extracellular structures that grow by taking component ions directly from the endolymph fluid that bathes them (Campana and Neilson 1985; Gauldie and Nelson 1990). Various hypotheses have been proposed to explain otolith growth and chemical composition, ranging from concentration gradients of ions within the endolymph fluid (Gauldie and Nelson 1990) to the composition of proteins that are thought to preclude and possibly control precipitation of calcium carbonate crystals (Dunkelberger et al. 1980; Pannella 1980). Both the ionic composition of endolymph fluid and the protein fiber matrix, coexisting with mineral crystals in otoliths, must be mediated or controlled by epithelial cells forming the inner layer of the endolymph sac (Dunkelberger et al. 1980; Saitoh and Yamada 1989). These conditions provide a mechanism for genetic control of otolith growth and allow for environmental effects on otoliths as well.

If the ionic composition of the endolymph is the major factor that controls the composition of the material being deposited on the otolith, then the chemical composition of the entire outer surface of an otolith should be similar, as it is immersed in a common fluid. This concept underlies most otolith microchemical studies that assume that otoliths are approximately radially symmetrical. However, we have found that the concentrations of strontium (Sr), sodium (Na), and potassium (K) in the otoliths of some Yukon River inconnu (*Stenodus leucichthys*) vary abruptly along segments that were precipitated at the same time. Herein, we present these findings, discuss their implications for models of otolith growth and elemental distribution, and suggest a procedure to avoid interpretive errors when examining otoliths that may have anomalous compositional patterns.

Materials and methods

Sagittal otoliths were removed from freshly killed inconnu, washed with water, and placed in coin envelopes to dry. Otoliths from 266 adult fish were prepared for optical examination. They were thin-sectioned in the transverse plane through the core (Secor et al. 1991) using a diamond grinding wheel, mounted on glass slides with Crystalbond Thermoplastic, and polished with 3-μm alumina powder. About 10% of these otoliths were affected by anomalies of the sort addressed in this paper, although we were not aware of this until well into the study.

Four of the affected otoliths were examined with a Cameca SX-50 electron microprobe equipped with four wavelength dispersive X-ray spectrometers. They were polished with 1-μm and 0.25-μm diamond paste and covered with a 30-nm layer of carbon. Line scans across the specimens were made with a 6-μm-diameter, 15-keV, 20-nA beam. Center to center distance between points was 10 μm. X-ray counts were collected for 25 s at each point in the transects. X-ray maps were made with a 200-nA beam, and counts were collected for 0.1 s at each point on a 9-μm grid across the otolith. Calcium, Sr, Na, and K X-rays were counted by the spectrometers. Two representative specimens are presented in this paper.

Four affected otoliths were examined with an ISI-40 scanning electron microscope (SEM). Sections were mounted to aluminum stubs and etched for about 2 min with a 0.1 M solution of ethylenediaminetetraacetic acid (EDTA) to provide surface relief to the crystal structure and growth increments (Gauldie and Nelson 1990). Etched sections were coated with a fine layer of gold prior to placement in the SEM. One representative specimen is presented in this paper.

Two affected and two unaffected otoliths were examined in a Rigaku Geigerflex X-ray diffractometer to determine their mineralogic composition. Otoliths were first examined optically to determine whether they were affected by the anomaly or not. Material from selected otoliths was crushed and ground to a fine powder with a mortar and pestle and fixed to a slide for examination in the X-ray diffractometer. It was not possible to reliably separate affected material from unaffected material in a given otolith specimen, so affected otoliths were examined as a mixture of both materials. One representative specimen from each type of otolith, affected and unaffected, is presented in this paper.

Results

Line scans from otoliths of inconnus 113 and 224 revealed regions with abnormally low X-ray counts of Sr, Na, and K. Despite low X-ray counts for Sr, Na, and K, affected areas had normal Ca X-ray counts (Fig. 1). Strontium maps of the otolith sections (Fig. 2) showed that the affected regions were discrete. The same pattern was seen in the distributions of Na and K, and the affected regions were identical within the resolution of the maps. Bands of enhanced Sr, arcing across the otolith of inconnu 224, end abruptly at the boundary between the two regions (Fig. 2*b*). The compositionally affected regions coincided directly with optically affected regions (Fig. 3*a*). Nonetheless, visible growth increment lines, altered though they were, continued through chemically affected regions.

SEM and X-ray diffraction analyses both suggested the presence of more than one type of crystalline mineral structure within affected otoliths. Optical images of affected otoliths and SEMs of etched otolith sections confirmed that the boundary between chemically affected and normal regions was abrupt and well defined (Fig. 3). The affected region appeared to be composed of very dense material, with a chaotic pattern of growth, as indicated by the etched lines in the image (Fig. 4*a*). By contrast, the unaffected region appeared to be composed of needlelike crystal flakes oriented perpendicular to regularly spaced growth increment lines (Fig. 4*b*). X-ray diffraction patterns from affected otoliths revealed the distinct presence of vaterite in addition to aragonite (Fig. 5), while unaffected otoliths had no sign of vaterite.

Discussion

Hypotheses about the mechanisms of otolith growth and the distribution of elements in otoliths, as discussed earlier, do not fully explain the findings of this study. The Sr maps (Fig. 2) and the SEM image (Fig. 3*b*) of affected and unaffected regions of otoliths show that there is no transition zone between the two, as would be expected if ion and pH gradients within a surrounding fluid were the sole controls of composition and structure.

The discovery that vaterite was present in affected otoliths (Fig. 5) suggests that it may be partly or entirely responsible

© 1999 NRC Canada

Fig. 1. Line scan graphs from the core to the margin of otoliths (*a*) 113 and (*b*) 224 showing relative X-ray counts for Ca, Sr, Na, and K, The anomalously low X-ray counts for Sr, Na, and K, which begin about midway along the scan for both specimens (arrows), alerted us to the phenomenon described in this paper. The discrete drops in Ca values are caused by cracks in the sample.

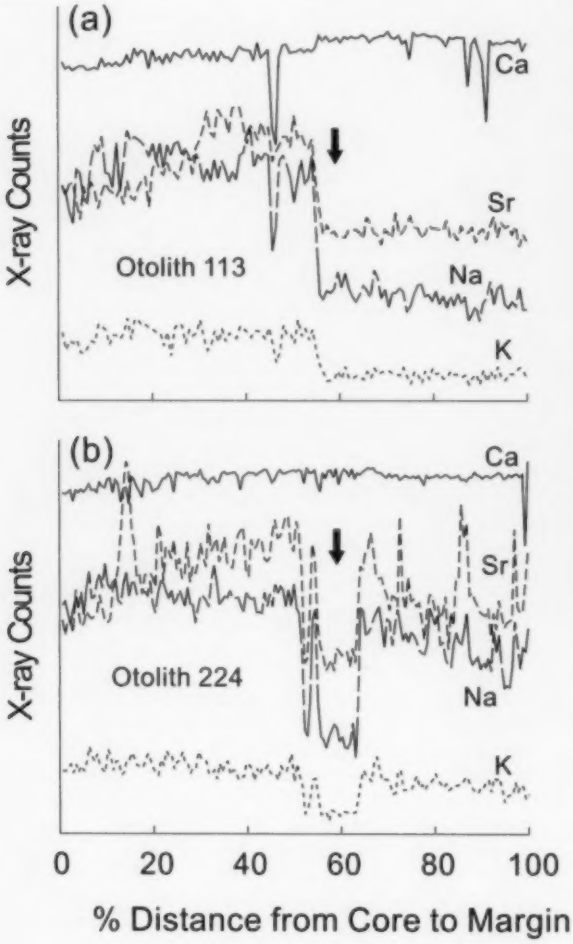
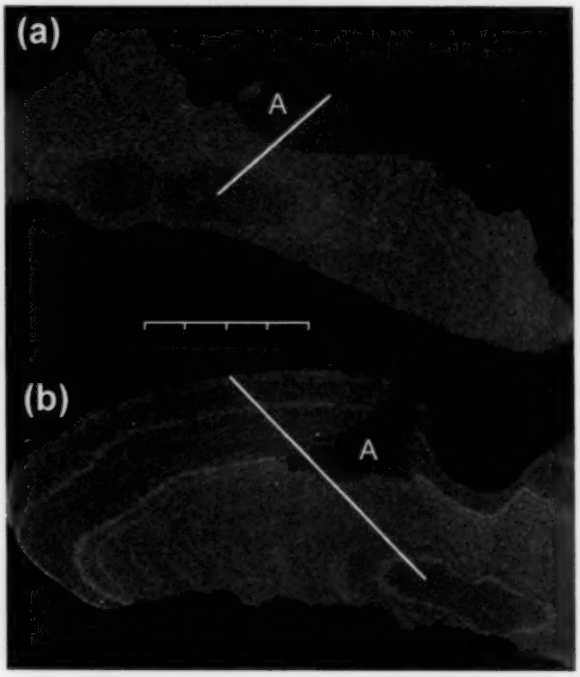


Fig. 2. Strontium X-ray maps of otoliths (*a*) 113 and (*b*) 224 showing the positions of the affected regions (A) and the approximate locations of the respective line scans from Fig. 1 across the otoliths. Regions of high Sr concentration are light, and those of low Sr concentration are dark. Scale bar = 1 mm.



for the anomalous chemical regions observed. However, the ability of vaterite to incorporate Sr and other cations is not well understood. It is possible that the substitution of Sr for Ca is less likely in vaterite than in aragonite (Lippmann 1973). Understanding that the crystal morph may influence the elemental composition of an otolith is of particular relevance to scientists using the distribution of elements associated with growth increments to infer life history events and migratory patterns.

To understand the problems associated with this phenomenon, we must review the interpretive logic used in anadromy studies based on otolith microchemistry. Secor et al. (1995) showed experimentally that Sr concentration in otoliths varied according to the salinity of the water that fish lived in when material was precipitated. Strontium concentration is relatively high in otolith material precipitated when a fish is in salt water. Strontium X-ray graphs created from line scans respond showing high Sr X-ray counts taken from that material. Conversely, Sr concentration is relatively low in otolith material precipitated when a fish is in freshwater, resulting in low Sr X-ray counts taken from that material. Given this principle, the Sr X-ray graph from the otolith of inconnu 224 (Fig. 1*b*), for example, would require the interpretation of the distinct drop in the Sr X-ray counts in midtransect (arrow) as a fish movement into an extremely low Sr environment. Examination of the Sr X-ray map for this otolith (Fig. 2*b*), however, reveals that the line scan simply crossed a region with an anomalously low concentration of Sr and that the remainder of the otolith appears to have a more normal distribution of Sr. It is clear that the chemistry of the affected region does not reflect the environmental history of this fish.

Proctor and Thresher (1998) reported that concentrations of Ca, Sr, Na, and K can be affected by contamination due to various otolith storage, handling, and preparation procedures. However, contamination is unlikely to have caused the elemental distribution patterns seen in this study. We observed distinct differences in the optical and crystalline appearance of anomalous regions relative to that of normal regions (Fig. 3), clearly ontogenetic effects, and the anomalous elemental distributions concurred exactly with these different regions.

The distinct crystal form found in affected regions of oto-

© 1999 NRC Canada

Fig. 3. (*a*) Photomicrograph of otolith 113 and (*b*) scanning electron micrograph of a similarly affected otolith showing the distinct difference between affected material (A) and unaffected material (U) and the discrete boundary between the two. Scale bar = 100 μm.

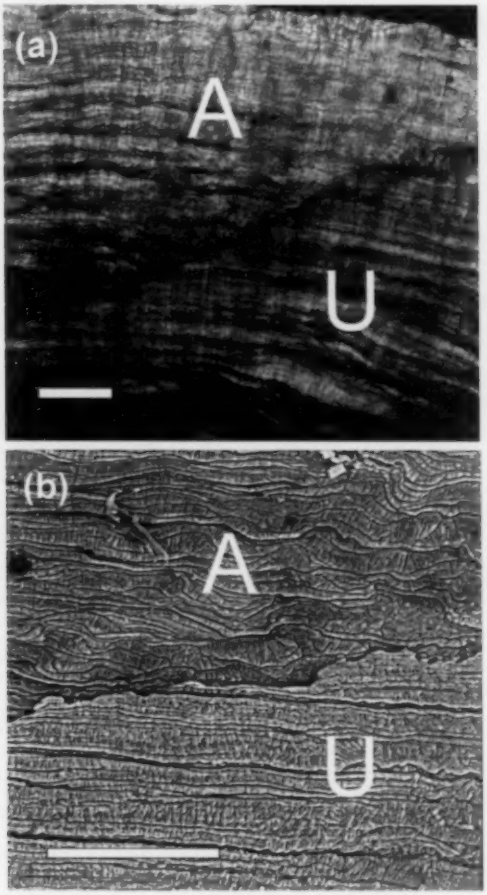
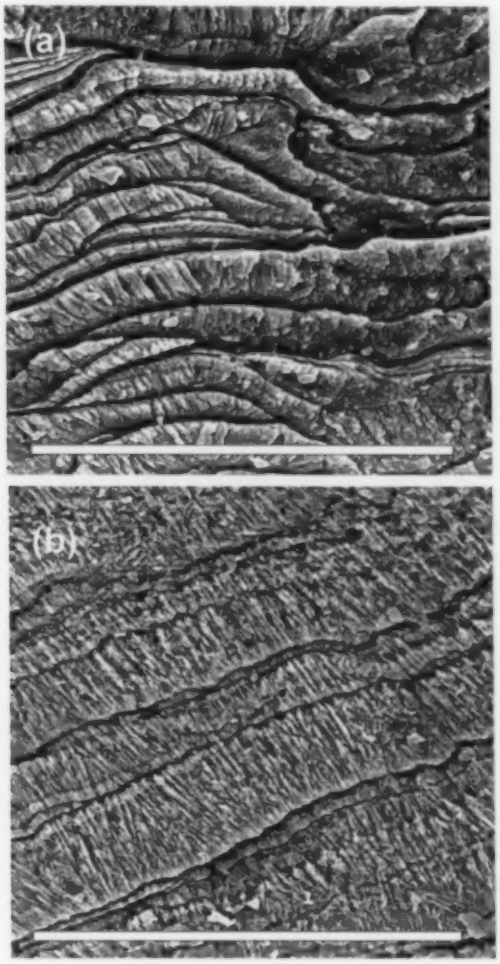


Fig. 4. Scanning electron micrographs of (*a*) affected and (*b*) unaffected regions of otoliths showing distinctly different fine structure of the mineral material. Scale bar = 100 μm.



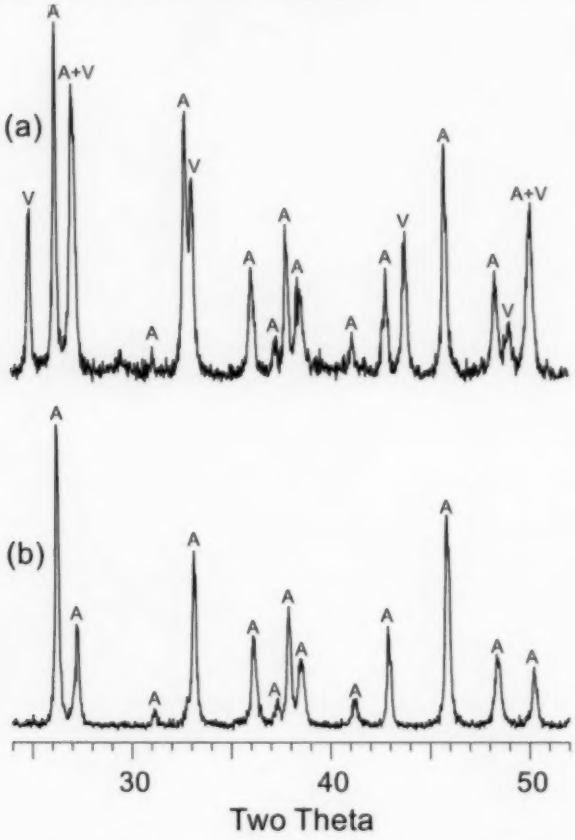
liths and the prominent vaterite peaks in the X-ray diffraction data from affected otoliths suggest that the affected areas are vaterite inclusions in otherwise aragonite otoliths. Gauldie (1993) identified polymorphic crystalline otoliths in many different families of fishes and discussed their qualities in some depth but did not speak to compositional variability between crystal forms. Campana (1983) briefly commented that vaterite inclusions in steelhead (*Oncorhynchus mykiss*) otoliths incorporated tetracycline similar to adjoining aragonite material. The affected regions in otoliths in our study are deficient in Sr, Na, and K relative to the unaffected regions. This finding suggests that the crystal structure may restrict the inclusion of these trace elements and perhaps others. We expect that the phenomenon should apply to the otoliths of other species as well.

The controlling mechanisms determining crystal form and growth within otoliths are unclear. Dunkelberger et al. (1980) identified material called subcupular meshwork extending from the cellular layer of the otolithic membrane to the otolith, containing fibers extending into the crystalline material of the otoliths. These fibers are presumably components of the proteinaceous matrix associated with otoliths. Dunkelberger et al. (1980) and Pannella (1980) suggested that the protein fibers provide a template facilitating precipitation. Furthermore, Pote and Ross (1991), in a study of otoconia from a wide range of vertebrates, determined that each crystal morph of biogenic calcium carbonate has unique proteins associated with it.

Because the proteins must be produced and extruded from the cells of the otolithic membrane, we can deduce that a gene, or genes, must switch production in some cells from proteins facilitating aragonite crystallization to those facilitating vaterite crystallization. This process is sufficient to explain the abrupt change from aragonite to vaterite across growth increments in an otolith and the change from an aragonite-only otolith in a young fish to one containing both aragonite and vaterite in an older fish. Both mineral forms would grow from ions in the common fluid bathing them, and both would be marked with

© 1999 NRC Canada

Fig. 5. Representative X-ray diffraction graphs of (*a*) affected and (*b*) unaffected otoliths showing the presence of aragonite peaks (A) in both and the presence of distinct vaterite peaks (V) in the graph from the affected otolith only.



common otolith growth increments. But physical properties unique to each mineral would determine optical appearances and specific elemental compositions.

Researchers must consider this phenomenon when they examine the distribution of elements across otolith growth increments to avoid confusing patterns caused by environmental variation with those caused by changes in otolith mineralogy. Detection of anomalous regions within otoliths could occur at any of several steps in the preparation and analytical process. Polymorphism can sometimes be detected by gross examination of whole otoliths. Campana (1983) described vaterite in polymorphic otoliths of steelhead as having a "glasslike appearance" (p. 1593), in contrast with the opaque white material normally seen in otoliths. This description mirrors our observations of polymorphic inconnu otoliths. Visual examination of thin-sectioned otoliths can reveal discontinuities that could be vaterite (Fig. 3*a*). Unusually abrupt changes in chemical composition along line scans across otoliths should be critically examined (Fig. 1). This requires knowledge about what is ordinary or expected, suggesting that interpretation of microchemical data from small sample sizes should be done cautiously. Finally, X-ray maps of otolith sections provide clear evidence of regions where common principles of otolith growth and elemental distribution fail (Fig. 2). X-ray line scans and maps are time-consuming and expensive, so the sooner in the process an odd otolith is identified and rejected, the more productive and efficient interpretive studies will be.

References

Babaluk, J.A., Halden, N.M., Reist, J.D., Kristofferson, A.H., Campbell, J.L., and Teesdale, W.J. 1997. Evidence for non-anadromous behaviour of Arctic charr (*Salvelinus alpinus*) from Lake Hazen, Ellesmere Island, Northwest Territories, Canada, based on scanning proton microprobe analysis of otolith strontium distribution. Arctic, **50**: 224–233.

Campana, S.E. 1983. Feeding periodicity and the production of daily growth increments in otoliths of steelhead trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Can. J. Fish. Aquat. Sci. **40**: 1591–1597.

Campana, S.E., and Neilson, J.D. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. **42**: 1014–1032.

Degens, E.T., Deuser, W.G., and Haedrich, R.L. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. **2**: 105–113.

Dunkelberger, D.G., Dean, J.M., and Norimitus, W. 1980. The ultrastructure of the otolithic membrane and otolith in the juvenile mummichog, *Fundulus heteroclitus*. J. Morphol. **163**: 367–377.

Farrell, J., and Campana, S.E. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. Comp. Biochem. Physiol. A, Comp. Physiol. **115**: 103–109.

Gauldie, R.W. 1993. Polymorphic crystalline structure of fish otoliths. J. Morphol. **218**: 1–28.

Gauldie, R.W., and Nelson, D.G.A. 1990. Otolith growth in fishes. Comp. Biochem. Physiol. A, Comp. Physiol. **97**: 119–135.

Kalish, J.M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. Mar. Ecol. Prog. Ser. **74**: 137–159.

Lippmann, F. 1973. Sedimentary carbonate minerals. Springer-Verlag, New York.

Lowenstam, H.A., and Weiner, S. 1989. On biomineralization. Oxford University Press, New York.

McFarlane, G.A., and Beamish, R.J. 1995. Validation of the otolith cross-section method of age determination for sablefish (*Anoplopoma fimbria*) using oxytetracycline. *In* Recent developments in fish otolith research. *Edited by* D.H. Secor, J.M. Dean, and S.E. Campana. University of South Carolina Press, Columbia, S.C. pp. 319–330.

Morrow, J.E. 1979. Preliminary keys to otoliths of some adult fishes of the Gulf of Alaska, Bering Sea, and Beaufort Sea. NOAA Tech. Rep. NMFS Circ. 420. U.S. Department of Commerce, Rockville, Md.

Mugiya, Y., and Tanaka, S. 1995. Incorporation of water-borne strontium into otoliths and its turnover in the goldfish *Carassius auratus*: effects of strontium concentrations, temperature, and 17β-estradiol. Fish. Sci. **61**: 29–35.

Mugiya, Y., and Uchimura, T. 1989. Otolith resorption induced by anaerobic stress in the goldfish, *Carassius auratus*. J. Fish Biol. **35**: 813–818.

Pannella, G. 1980. Growth patterns in fish sagittae. *In* Skeletal growth of aquatic organisms. *Edited by* D.C. Rhoads and R.A. Lutz. Plenum Publishing Corp., New York. pp. 519–560.

Platt, C., and Popper, A.N. 1981. Fine structure and function of the ear. *In* Hearing and sound communication in fishes. *Edited by*

© 1999 NRC Canada

W.N. Tavolga, A.N. Popper, and R.R. Fay. Springer-Verlag, New York. pp. 3–36.

Pote, K.G., and Ross, M.D. 1991. Each otoconia polymorph has a protein unique to that polymorph. Comp. Biochem. Physiol. B, Comp. Biochem. **98**(2/3): 287–295.

Proctor, C.H., and Thresher, R.E. 1998. Effects of specimen handling and otolith preparation on concentration of elements in fish otoliths. Mar. Biol. **131**: 681–694.

Sadovy, Y., and Severin, K.P. 1994. Elemental patterns in red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto Rico reflect growth rate, not temperature. Can. J. Fish. Aquat. Sci. **51**: 133–141.

Saitoh, S., and Yamada, J. 1989. Ultrastructure of the saccular epithelium and the otolithic membrane in relation to otolith growth in tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). Trans. Am. Microsc. Soc. **108**: 223–238.

Secor, D.H. 1992. Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass *Morone saxatilis*. Fish. Bull. U.S. **90**: 798–806.

Secor, D.H., Dean, J.M., and Laban, E.H. 1991. Manual for otolith removal and preparation for microstructural examination. Baruch Press, University of South Carolina, Columbia, S.C.

Secor, D.H., Henderson-Arzapalo, A., and Piccoli, P.M. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol. Ecol. **192**: 15–33.

Tzeng, W. 1996. Effects of salinity and ontogenetic movements on strontium:calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. J. Exp. Mar. Biol. Ecol. **199**: 111–122.

Tzeng, W., Severin, K.P., and Wickstrom, H. 1997. Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. Mar. Ecol. Prog. Ser. **149**: 73–81.

© 1999 NRC Canada

New perspectives on the growth and longevity of the European lobster (*Homarus gammarus*)

M.R.J. Sheehy, R.C.A. Bannister, J.F. Wickins, and P.M.J. Shelton

Abstract: The natural rate of lipofuscin accumulation in an eyestalk ganglion was determined from microtagged European lobsters, *Homarus gammarus*, of known age, recaptured from the Yorkshire fishery (United Kingdom). This calibration, in combination with supporting data from shorter-lived astacideans (freshwater crayfish), was used to age wild lobsters from the fishery. A unique perspective of age-at-size in a clawed-lobster population was obtained, which circumvented some difficulties associated with conventional methods for estimating generalized growth and natural mortality. The exceptional ages attained by some of the largest lobsters (males: average 31 years, maximum 42 ± 5 years; females: average 54 years, maximum 72 ± 9 years) are explained by ageing theory, indicate natural mortality rates, M, of 0.15 and 0.08 for males and females, respectively, and point to the existence of an offshore refuge. Age-at-size is highly variable: at least seven year-classes enter the fishery at 85 mm carapace length. This limits resolution of annual cohorts in size compositions, complicates development of recruitment indices, and may explain past size composition stability. The new age–length data suggest potential selective fishing impacts and past early recruitment variations. The study highlights the need for age data in order to obtain accurate crustacean stock assessments.

Résumé : On a déterminé le taux naturel d'accumulation de lipofuscine dans un ganglion du pédoncule oculaire de homards européens, *Homarus gammarus*, munis de micromarques et d'âge connu, recapturés dans la pêche du Yorkshire (R.-U.). Cet étalonnage, combiné à des données connexes tirées d'astacidés (écrevisses), organismes qui vivent moins longtemps, a été utilisé pour âger des homards sauvages pêchés. On a ainsi obtenu une nouvelle image de l'âge selon la longueur dans une population de homard européen qui permet de lever certaines difficultés associées aux méthodes classiques d'estimation de la croissance globale et de la mortalité naturelle. La longévité exceptionnelle de certains des plus gros homards (mâles : moyenne de 31 ans, max. de 42 ± 5 ans; femelles : moyenne de 54 ans, max. de 72 ± 9 ans) s'explique par la théorie du vieillissement, se solde par des taux de mortalité naturelle, M, de 0,15 et 0,08 chez les mâles et les femelles, respectivement, et laisse croire en l'existence d'un refuge en haute mer. L'âge selon la longueur est très variable : au moins 7 classes d'âge sont recrutées dans la pêche avec une carapace de 85 mm. Cela limite notre capacité d'établir avec précision des cohortes annuelles selon les tailles, complique l'établissement d'indices de recrutement, et peut expliquer la stabilité passée des compositions par taille. Les nouvelles données sur les âges selon la longueur laissent croire en l'existence d'impacts possibles de la pêche sélective et de variations passées dans le recrutement des jeunes homards. Cette étude met en évidence le fait qu'on doit disposer de données sur les âges pour obtenir des évaluations précises des stocks de crustacés.

[Traduit par la Rédaction]

Introduction

The description of growth and its variability and the quantification of natural mortality continue to pose difficulties for the modelling and assessment of lobster populations by length-based methods (e.g., length cohort analysis (LCA), Jones 1974) (Addison 1997).

In lieu of age–length data, a conventional approach for generating von Bertalanffy growth parameters for lobster populations has been to fit Ford–Walford lines through plots of "anniversary" growth increments derived from tag recaptures (Hancock and Edwards 1967). One difficulty with this approach is the varying catchability of lobsters caused by uneven feeding motivation over the moult cycle. Increased feeding activity in recently moulted lobsters means that they would be expected to enter baited traps more readily than nonmoulters (Cobb 1995), with the consequence that average annual growth is overestimated. The extended moulting season for lobsters may be difficult to avoid when undertaking tag–recapture programs (Hancock and Edwards 1967). A

Received December 21, 1998. Accepted June 16, 1999
J14940

M.R.J. Sheehy[1] **and P.M.J. Shelton.** Biology Department, Adrian Building, University of Leicester, University Road, Leicester, LE1 7RH, U.K.
R.C.A. Bannister. The Centre for Environment, Fisheries and Aquaculture Science, Lowestoft Laboratory, Pakefield Road, Lowestoft, NR33 OHT, U.K.
J.F. Wickins. The Centre for Environment, Fisheries and Aquaculture Science, Conwy Laboratory, Benarth Road, Conwy, LL32 8UB, U.K.

[1]Author to whom all correspondence should be addressed. e-mail: mrjs2@le.ac.uk

© 1999 NRC Canada

further problem is that, in heavily exploited inshore fisheries, while the probability of tagged, legal-sized individuals remaining at liberty for more than a couple of years is low, the largest lobsters may have intermoult periods longer than this. This makes defining the upper end of the growth curve exceedingly difficult (Fogarty 1995).

Without data for the preexploited condition, estimates of instantaneous natural mortality rate, M, for fished lobster stocks have been made indirectly by subtracting the estimated rate of fishing mortality, F, from that of total mortality, Z; these latter coefficients having been derived from tag–recapture and size–frequency data. Ennis (1979) discussed several potential sources of error that are inherent in this approach. More direct methods for estimating M empirically, from longevity data, have been developed (Hoenig 1983), but, in the absence of ageing criteria, the natural life span of clawed lobsters has long remained a source of speculation (Wolff 1978), scientifically informed only by projections from the widely varying results of growth studies.

While potential biases are inevitable in any tag- or trap-based sampling of lobster populations (Krouse 1989), including those in the present study, the use of biological markers can overcome some of the difficulties. We previously described age-dependent deposition of autofluorescent lysosomotropic compounds (lipofuscin) in the eyestalk of the European lobster, *Homarus gammarus* (Sheehy et al. 1996). As a cellular index of biological age, accumulating independently of growth and the moult cycle in all members of the population, not just a small, tagged subpopulation, lipofuscin provides data that circumvent certain problems associated with conventional procedures for estimating generalized growth and M. Here, we present the first age–length analysis of a wild clawed-lobster population, that of *H. gammarus* from the Yorkshire fishery in the United Kingdom.

Materials and methods

Lobster samples

Eye samples from three groups of *H. gammarus* were used for this study. The first group was a reference sample of 53 microtagged lobsters (28 females and 25 males) ranging in known age from 4.3 to 8.4 years (± 1.3 months). These had been randomly selected out of 1991–1992 recaptures from a stock enhancement trial conducted in Bridlington Bay (53°55′N, 00°05′W), an area of the Yorkshire inshore fishery on the northeast coast of England (Bannister et al. 1994). They had been hatchery reared at ~16–22°C for 4–6 months prior to being released into the area between 1984 and 1988. Average annual sea surface temperature here (Spurn Point, 1980–1994) was 10.0°C, with February and August averages of 4.3 and 16.6°C, respectively.

The second group consisted of a stratified sample of 253 naturally settled lobsters (116 females and 137 males) from the Yorkshire inshore fishing grounds. For this sample, roughly even coverage of the predominant trappable size groups was sought by collection of about 50 individuals from each of the 60–70, 70–80, 80–90, and 90–100 mm orbital carapace length (OCL) classes, with a further 50 individuals of >100 mm OCL. The sample was obtained between August and October 1996 from either a commercial wholesaler at Bridlington or, for individuals smaller than the 1996 minimum landing size (MLS), 85 mm OCL, by special collection from a local fishing vessel.

The third group consisted of 40 wild lobsters (19 females and 21 males) from the largest size groups present in the Yorkshire fishery (>130 mm OCL). These were obtained from commercial wholesalers at Bridlington and Scarborough in July–August 1997 by actively searching commercial landings and were considered to have originated primarily from the offshore fishery, 8–20 km from the coast. For comparison, two unusually large lobsters from the Cornish fishery in southwest England, a male of 166 mm OCL (Sheehy et al. 1996) and a female of 157 mm OCL, were obtained from a commercial wholesaler in Plymouth in September 1995 and October 1996, respectively.

Shell condition

Shell condition was used as an indicator of recent moulting (Ennis 1977). Lost or damaged appendages and other old wounds were noted and the proportion of shell coverage with epibionts was scored as follows: 0 (~0–5% of surface area), 1 (~5–25%), 2 (~25–50%), 3 (~50–75%), or 4 (~75–100%).

Lipofuscin measurement

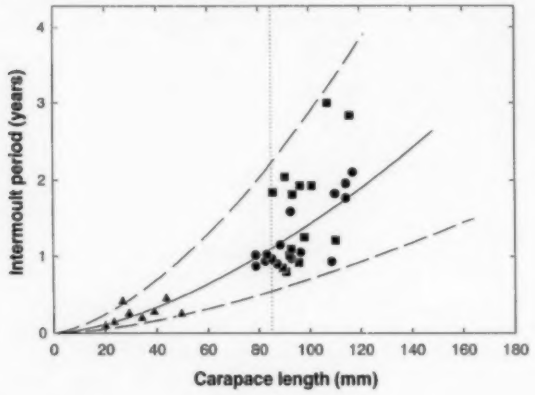
Lipofuscin concentration in globuli cell cluster A of the eyestalk medulla terminalis (MTALC) was quantified as a volume fraction (%VF) using the confocal microscopic and image analysis methods described in Sheehy et al. (1996) and the references cited therein. The only refinement to this protocol was that the central tendency of replicate lipofuscin measurements for each lobster was mathematically defined with a weighted geometric average (Sheehy et al. 1998) rather than an arithmetic average. All measurements on reference samples were undertaken blindly, i.e., without knowledge of the age of the individual.

Age prediction and error

The relationship between MTALC and chronological age in the reference lobsters was described by a linear regression, which was then used to back-calculate chronological ages for the wild lobsters from their MTALCs. In many cases, this necessitated extreme extrapolation because the available reference data spanned only the lower 6% of the total range of MTALCs found in the wild lobsters. Uncertainty across the entire range of age predictions was assessed as follows. (*i*) Inverse regression procedures (nonreplicated *Y*s) were used to obtain 95% confidence intervals for age predictions generated from the reference data (Sokal and Rohlf 1981, p. 498). (*ii*) A distribution-free bootstrap analysis (S-PLUS v. 4.5, MathSoft Inc., Cambridge, Mass.) was used to test the effect of sampling on the prediction regression. Paired observations from the reference data were randomly subsampled to $n = 53$ and the regression model refitted in each of 1000 iterations so as to obtain 95 percentiles for the regression coefficients. (*iii*) An empirical approach was used to infer potential errors in the age extrapolations. This was based on comparison with previous data for the shorter-lived freshwater crayfish *Cherax quadricarinatus* (Sheehy et al. 1994) and *Pacifastacus leniusculus* (Belchier et al. 1998) growing under natural temperature conditions. These data cover a greater relative proportion of the total life span than those obtained for *H. gammarus*. The comparison assumes that, while rate of lipofuscin accumulation (and physiological ageing) is inversely proportional to species-specific longevity, there is a degree of interspecific uniformity, both in the temporal pattern of lipofuscin accumulation and in the final lipofuscin loading reached by a particular cell type during senescence. These assumptions are supported by the results of some previous studies that span diverse animal groups (see Sohal 1981; Sheehy 1992; Sheehy et al. 1995, 1996), and further evidence is provided by the present results (see Discussion). To facilitate comparisons, the originally published lipofuscin concentrations for *C. quadricarinatus* (arithmetic averages) were refined to weighted geometric averages. Also, because

© 1999 NRC Canada

Fig. 1. Relationship between OCL and intermoult period for laboratory-housed *H. gammarus* from Hepper (1978). Original data replotted on linear scales: solid line, best-fitting regression; dashed lines, 95% PL; squares, males; circles, females; triangles, juveniles; vertical dotted line, 1996 MLS.



lipofuscin accumulates sufficiently rapidly in this species to permit detection of seasonal oscillations in deposition rate that are not readily resolvable in longer-lived species, the data were linearized by transformation of the X (age) variable. Both X and Y variables in both freshwater crayfish data sets were further root transformed to achieve normality and homoscedasticity prior to regression analysis. Ninety-five percent confidence limits (CL) for age predictions from the crayfish data sets were then calculated (as plus and minus percentages of the prediction) using a procedure similar to that described in method *i* but for replicated Ys. To apply this information to *H. gammarus* age extrapolations, these percentages were transposed to the ages predicted from the equivalent MTALCs in the lobster and then converted back to absolute ages. All data were detransformed for graphing.

Estimation of *M*

The average ages of the largest male and female lobsters obtained from sorting of offshore Yorkshire catches (150–170 mm OCL) were arbitrarily taken as conservative indicators of the natural life span (T_{max}) of Yorkshire lobsters (Hoenig 1983). From these averages, M was calculated using Hoenig's (1983) empirically derived relationship, which is based on data from 134 minimally exploited populations of 79 fish, mollusc, and cetacean species ($r^2 = 0.82$):

$$\ln(M) = 1.44 - 0.982 \ln(T_{max})$$

Fitting growth curves

Central tendency in the scattered age–length data was first elucidated using LOWESS smoothing (locally weighted regression) (SigmaPlot v. 4.0, SPSS Inc., Chicago, Ill.) with relatively small smoothing factors of 0.06–0.09. The von Bertalanffy equation and various other functions were then fitted to the smoothed data using iterative least-squares methods (Marquardt–Levenberg algorithm, SigmaPlot v. 4.0). Goodness-of-fit was assessed by eye and the optimal model selected for description of generalized growth. For curve fitting, size was treated as the independent variable and age as the dependent variable. This approach removed some difficulties in the determination of mean size-at-age arising from size selectivity in the catch. For instance, capture of lobsters below about 55 mm OCL, which would otherwise have formed a substantial component of the younger age groups in the sample, was virtually precluded by trap design and cryptic juvenile behaviour.

Estimation of average and extreme sizes-at-age from moult increment and moult frequency data

For comparison with the lipofuscin-based approach, generalized growth and individual variation were assessed from previously published moult increment (MI) and moult frequency (MF) data for *H. gammarus* (Hepper 1967, 1978), which were obtained from Yorkshire tag recaptures and laboratory rearing experiments under ambient temperature conditions, respectively. Estimation of the slowest expected growth pattern was based on sustained moulting at Hepper's minimum recorded MI (4 mm OCL) and most infrequent moulting, as defined by the upper 95% prediction limit (PL) for the relationship between OCL and intermoult period (Fig. 1):

$$\ln(Y) = 1.57 \ln(X) - 0.968$$

where X is OCL (millimetres) and Y is intermoult period (days). This equation represents Hepper's (1978) complete data set, not that omitting 2- to 3-year intermoults. Maximum intermoult period (not estimable from Hepper's data range) was constrained to a biologically plausible maximum of 4 years (Waddy et al. 1995). Estimation of the fastest possible growth trajectory was based on sustained moulting at Hepper's maximum observed MI (13 mm OCL), in combination with the most frequent moulting observed, as defined by the lower 95% PL for the relationship between intermoult period and OCL. Average growth formats were approximated for males and females using the mean intermoult period-at-size relationship above, in combination with the reported average MIs for males (9.8 mm OCL) and females (8.4 mm OCL).

Results

Growth and lipofuscin accumulation in known-age lobsters

There was a significant linear relationship between MTALC (%VF) and known chronological age (t, years) in the reference lobsters ($p < 0.0001$, $r^2 = 0.31$) (Fig. 2*a*) as follows:

$$t = \frac{\text{MTALC} + 0.131}{0.105}$$

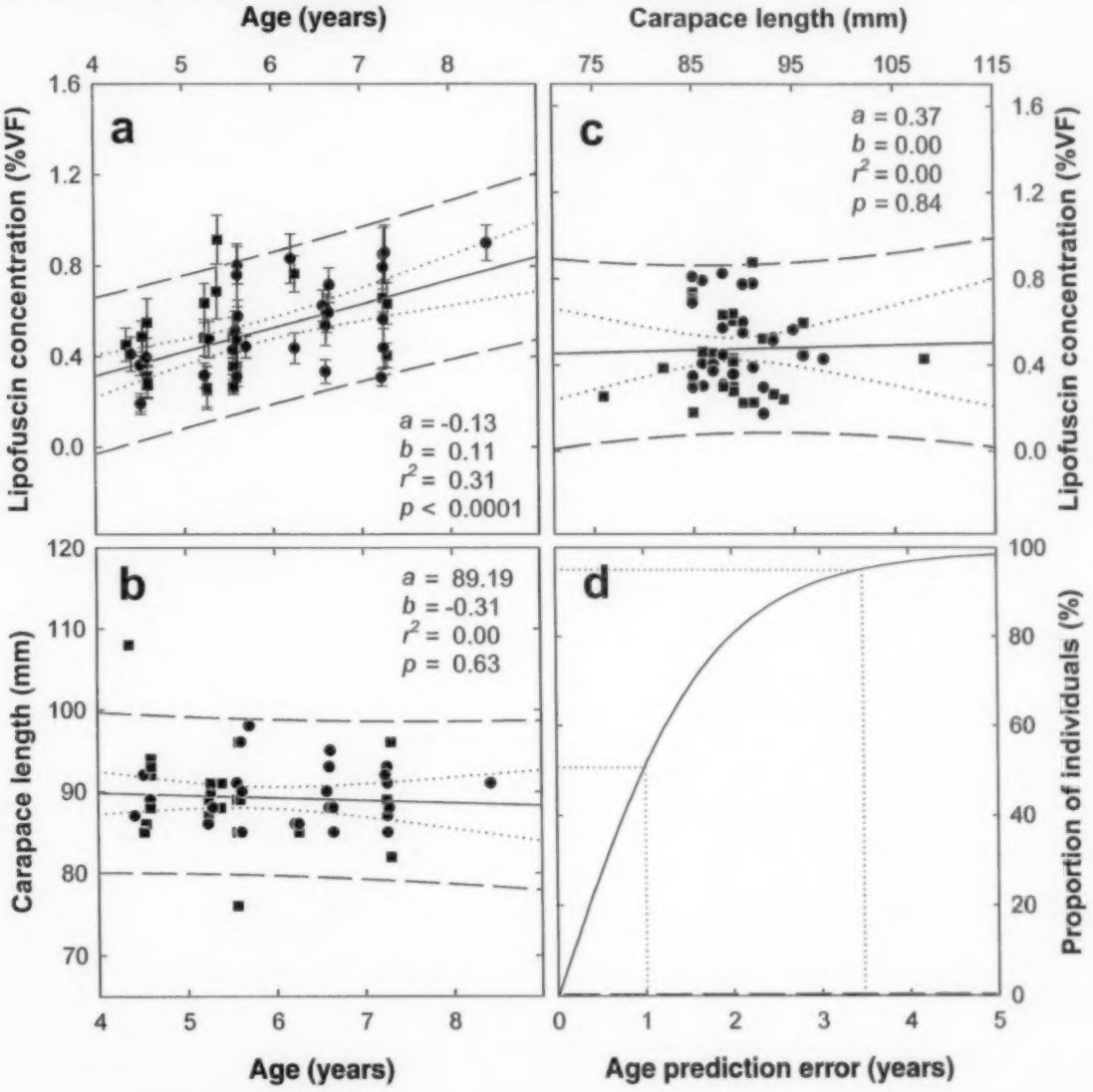
There were no significant sexual differences in MTALC after allowing for the effects of differences in age (two-way ANOVA, $p = 0.77$). There was also no relationship between OCL and either lobster age (Fig. 2*b*) or MTALC (Fig. 2*c*) in the reference sample.

Age prediction error

The nonsignificant age–length relationship within the range of the reference sample meant that OCL could not be used to predict age. However, age predictions were possible using MTALC, the mean residual error around the predictive regression being 1.2 years. In the range of the reference sample, MTALC correctly ages 51% of lobsters to within ±1 year and 95% to within ±3.5 years (Fig. 2*d*). The further the extrapolation beyond the range of the reference data, the larger the uncertainty in age predictions (Figs. 3*a*–3*c*). For the most extreme extrapolation, based on the highest MTALC encountered in the wild Yorkshire lobsters (7.4% VF, predicted age = 72 years), the 95% prediction interval was 52–120 years where extrapolation was based on the *H. gammarus* reference sample alone to about 63–82 years by inference from the more extensive freshwater crayfish data sets. The latter produced very similar 95% confidence intervals for upper-end age estimates, both averaging around ±12% of the prediction. Note that the within-age-group vari-

© 1999 NRC Canada

Fig. 2. Relationship between age and (*a*) MTALC and (*b*) OCL and between (*c*) OCL and MTALC for microtagged known-age *H. gammarus* recaptured from the Yorkshire fishery. Circles, females; squares, males; solid, dotted, and dashed lines represent best-fitting regressions and their 95% CL and PL, respectively. Note that for the purpose of depicting their 95% confidence intervals, lipofuscin concentrations in Fig. 2*a* are shown as arithmetic averages rather than weighted geometric averages. This has negligible effect on the fitted linear regression statistics, which are also shown. (*d*) Relationship between potential age prediction error (confidence interval for age estimates) and the corresponding proportion of individuals in a sample that will be aged correctly to within that level of error, based on the results from the tag recaptures shown in Figs. 2*a* and 2*b*. Solid and dashed (on *x*-axis) lines represent the relationships for MTALC-based and OCL-based age predictions, respectively.



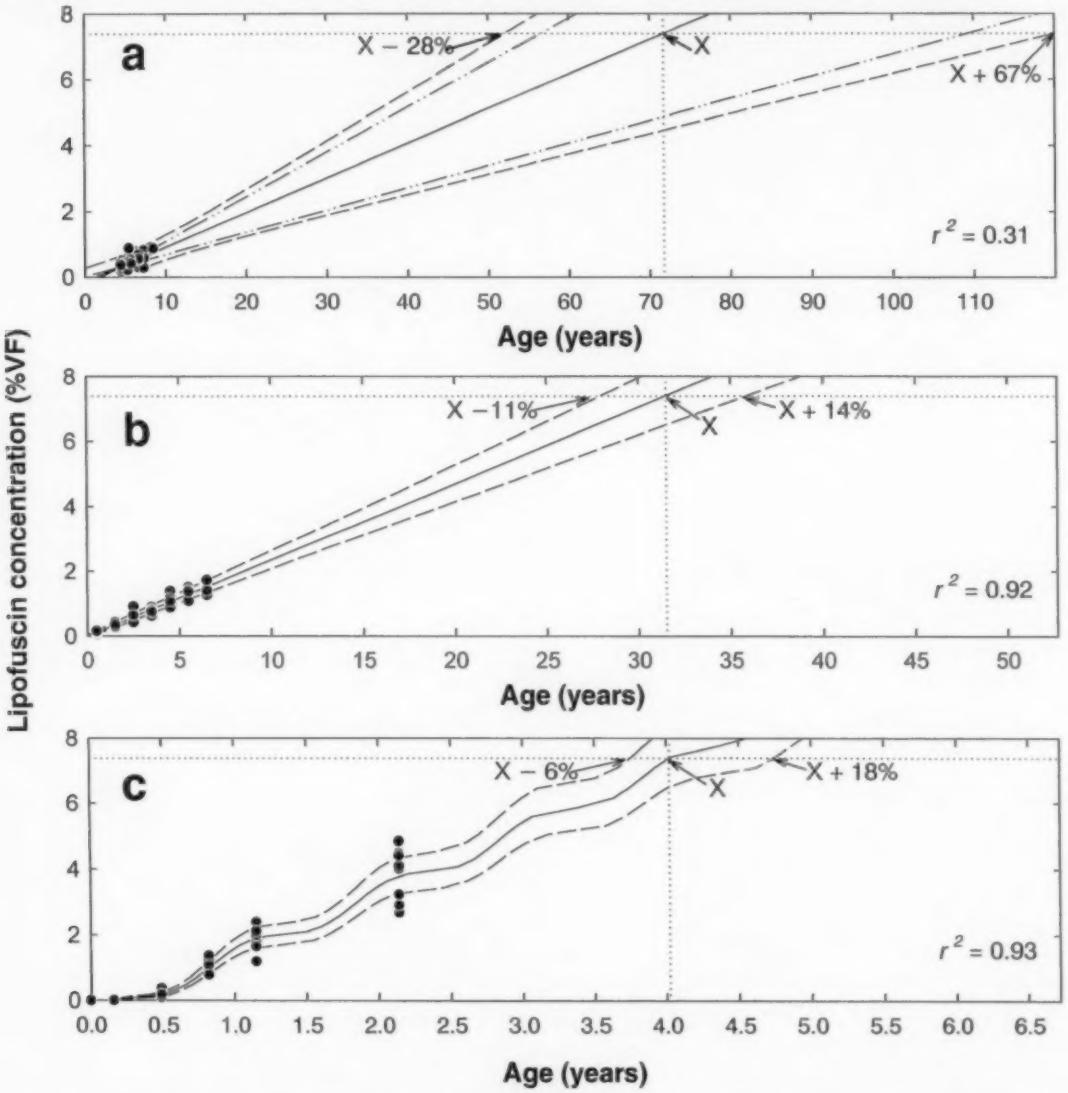
ance in the untransformed lipofuscin concentrations across the three species at comparable lipofuscin levels was statistically homogeneous (Levene median test, $p > 0.05$) despite widely differing life spans and accumulation rates. All methods of CL calculation suggested that the oldest lobsters sampled in the present study were in excess of 50 years of age.

Maximum age, *M*, and reproductive life span

The oldest female lobsters sampled were substantially older than the oldest males (Fig. 4*a*). Lipofuscin-based age estimates for the two largest female lobsters (151 and 169 mm OCL) were extreme at 72 ± 9 years (age prediction and its inferred 95% CL from method *iii* above) and 61 ± 7 years, respectively. The oldest male sampled (150 mm OCL), although not the largest, was estimated to be 42 ± 5 years old. The average ages of the largest males and females sampled (150–170 mm OCL) were 31 and 54 years, respectively. These mean ages produced preliminary *M*s of 0.15 (14%/year) and 0.08 (8%/year) for

© 1999 NRC Canada

Fig. 3. Confidence intervals for *H. gammarus* age predictions based on extrapolations beyond the reference sample range. Estimates are based on (*a*) the present reference data alone or on inference from more extensive previous data sets for the shorter-lived freshwater crayfish (*b*) *P. leniusculus* (Belchier et al. 1998) and (*c*) *C. quadricarinatus* (Sheehy et al. 1994). Horizontal dotted line, maximum recorded crustacean neural lipofuscin concentration (7.4% VF); vertical dotted line, estimated age at putative maximum lipofuscin concentration; solid line, fitted regression (and bootstrap median in Fig. 3*a*); dashed–dotted lines (in Fig. 3*a* only), bootstrap 95 percentiles for fitted regression; dashed lines, 95% CL for age predictions by inverse regression. Annotations at the upper ends of the regression lines refer to the most extreme age extrapolations, *X*, and their 95% CL as plus and minus percentages of *X*.



males and females, respectively. The estimated ages of gravid females in the sample ranged from 6.9 to 49.5 years (Fig. 4*a*), indicating a potential reproductive life span for *H. gammarus* in excess of 40 years.

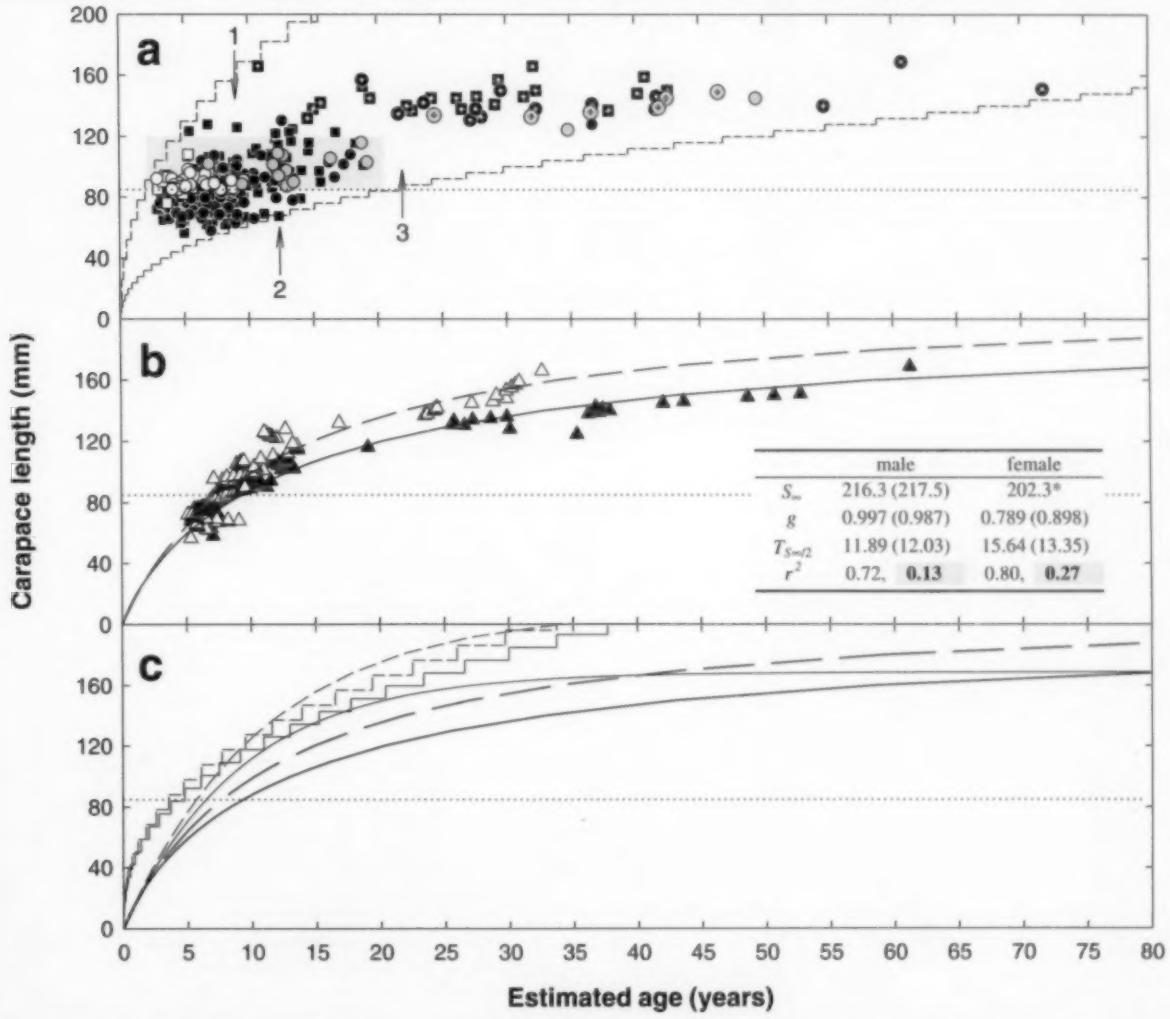
Growth variation in wild lobsters

Variation in size-at-age was relatively wide but appeared to be reduced in the older age groups. By inference from Fig. 4*a*, small untrappable lobsters (<55 mm OCL) are a component of all age groups younger than about 7 years. In the age range 5–12 years, a few younger, relatively fast-growing males were apparent in the top part of the scatter distribution (Figs. 4*a* (arrow 1) and 5*a*). Size variation was greater in males than in females (see r^2 values in Fig. 4).

Variation in age-at-size was large in all catchable size groups and increased with size (Figs. 4*a* and 5*a*). The smallest lobsters sampled (55–70 mm OCL) included relatively fast-growing estimated 3-year-olds through to stunted individuals estimated to be as old as 12 years. The 80–89.9 mm OCL class of sampled lobsters, spanning the MLS, con-

© 1999 NRC Canada

Fig. 4. Relationship between OCL and lipofuscin-estimated age for sampled wild Yorkshire *H. gammarus*. (*a*) Raw estimated age–length data: squares, males; circles, females; open symbols, Yorkshire reference lobsters plotted as size versus lipofuscin back-calculated age; sold black symbols, Yorkshire lobsters collected in 1996 and originating primarily from inshore fishing grounds with gravid females shown as solid grey circles; black symbols with cross, Yorkshire lobsters collected in 1997 and originating primarily from offshore fishing grounds with gravid females shown as grey circles with cross; black symbols with dot, Cornish lobsters; stepped dashed lines, estimated growth extremes based on 95% PL for intermoult period at size (Fig. 1) and MI extremes of 4 and 13 mm OCL (Hepper 1967); horizontal dotted line, 1996 MLS; arrows, see Results and Discussion sections; shaded box, size range of lobsters encompassing >98% of all legal-sized landings in the Yorkshire fishery in 1996. (*b*) LOWESS-smoothed estimated age–length data from Fig. 4*a*, excluding Cornish lobsters: open triangles, males; black triangles, females; dashed and solid lines, best-fitting logistic models for males and females, respectively. Regression coefficients for the models are shown in the inset. Values in parentheses refer to the same model when fitted directly to the raw data in Fig. 4*a*; the asterisk denotes the value constrained to the OCL equivalent of the female asymptotic mass estimate of 4763.9 g (mass–length conversion from Bannister et al. 1983). Coefficients of determination refer to the raw data in Fig. 4*a*, the first value for each sex representing the whole age–length range, and the second (shaded) that within the shaded size range in Fig. 4*a*, i.e., the majority of the catch. (*c*) Comparison of generalized growth models from the present study (thick lines) with conventional von Bertalanffy models for the Yorkshire fishery (Bannister et al. 1983) and predicted growth based on average moult format (Fig. 1) (Hepper 1967, 1978) (thin curved and stepped lines, respectively). Dashed and solid lines, males and females, respectively.

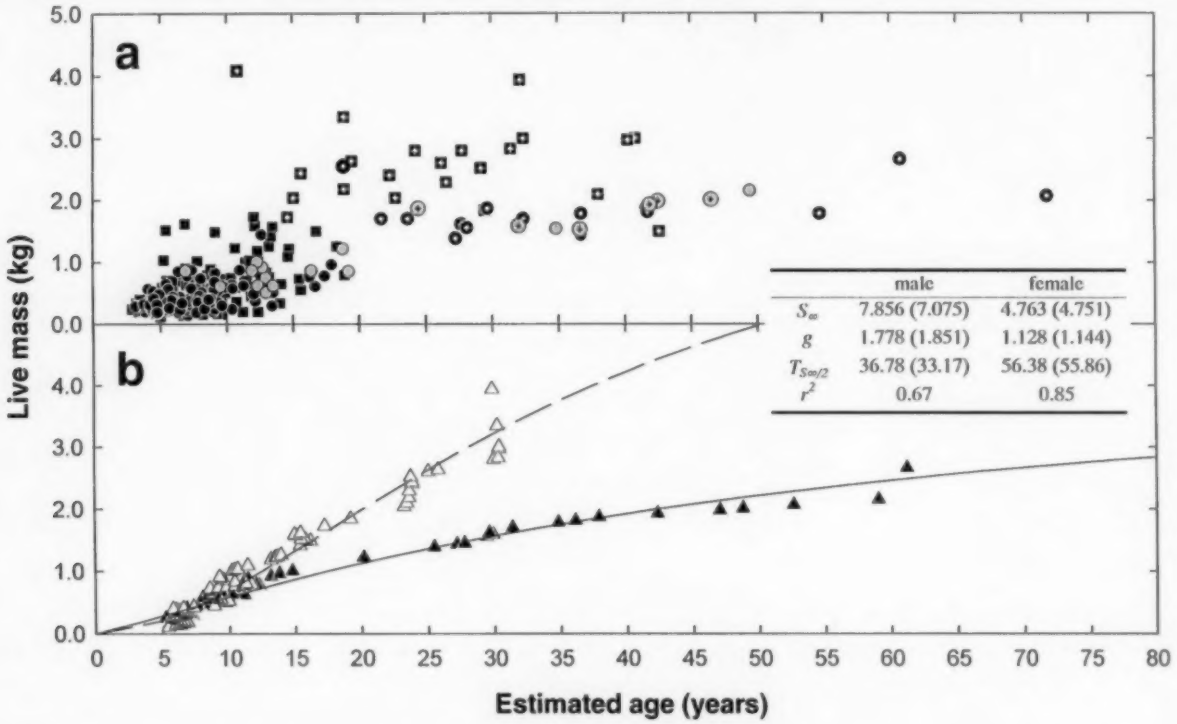


tained males estimated to be 3.5–12.9 years old and females 4.1–13.0 years old. Age difference in similarly sized lobsters was extreme above 130 mm OCL and about 1.5 kg body mass, especially for females: two individuals, 150 and 151 mm OCL, respectively, were estimated to differ in age by more than 40 years, far more than any passive effect of age extrapolation error.

The pattern of individual variation in lipofuscin-estimated

© 1999 NRC Canada

Fig. 5. Relationship between live mass and estimated age for sampled wild Yorkshire *H. gammarus*. (*a*) Raw estimated age – live mass data: squares, males; circles females; solid black symbols, Yorkshire lobsters collected in 1996 and originating primarily from inshore fishing grounds with gravid females shown as solid grey circles; black symbols with cross, Yorkshire lobsters collected in 1997 and originating primarily from offshore fishing grounds with gravid females shown as grey circles with cross; black symbols with dot, Cornish lobsters. Live mass data were not available for the reference lobsters. (*b*) LOWESS-smoothed data from Fig. 5*a*, excluding Cornish lobsters: open triangles, males; black triangles, females; dashed and solid lines, best-fitting logistic models for males and females, respectively. The regression coefficients for the models are shown in the inset. Values in parentheses and r^2 coefficients refer to the same model when fitted directly to the raw data in Fig. 5*a*.



age–length was completely encompassed within the extremes of growth predicted from earlier moult data (Fig. 4*a*), particularly in the region of the highest data point density (60–110 mm OCL). The pattern suggests a relative paucity of the largest individuals in all recruiting age groups (Fig. 4*a*, arrow 1). There was also a conspicuous paucity of lobsters from the inshore fishery older than about 20 years of age (Fig. 4*a*, arrow 3). This is very close to the age after which there is predicted to be total recruitment to the fishery at 85 mm OCL, based on the lipofuscin-estimated age–length variation and the slowest predicted growth extreme from the moult data. A cluster of 18 data points (Fig. 4*a*, arrow 2) represents lobsters of a wide size range (65–130 mm OCL), but all about 12–13 years old, and including several gravid females. The size range that encompassed more than 98% of legal-sized landings in the Yorkshire fishery in 1996 (85–120 mm OCL) contained lobsters estimated to be about 4–20 years old (Fig. 4*a*, shaded area). Over this range, the relationship between estimated age and size was poor for both sexes (males: r^2 = 0.13; females: r^2 = 0.27). The two large Cornwall lobsters sampled (Fig. 4*a*) were both estimated to be younger than all Yorkshire lobsters of comparable size; however, these lobsters still fell within the ranges predicted from the moult data.

Generalized growth

The data for OCL and live mass of lobsters of both sexes (Figs. 4*b* and 5*b*) were optimally fitted by a single inverse logistic model of the form

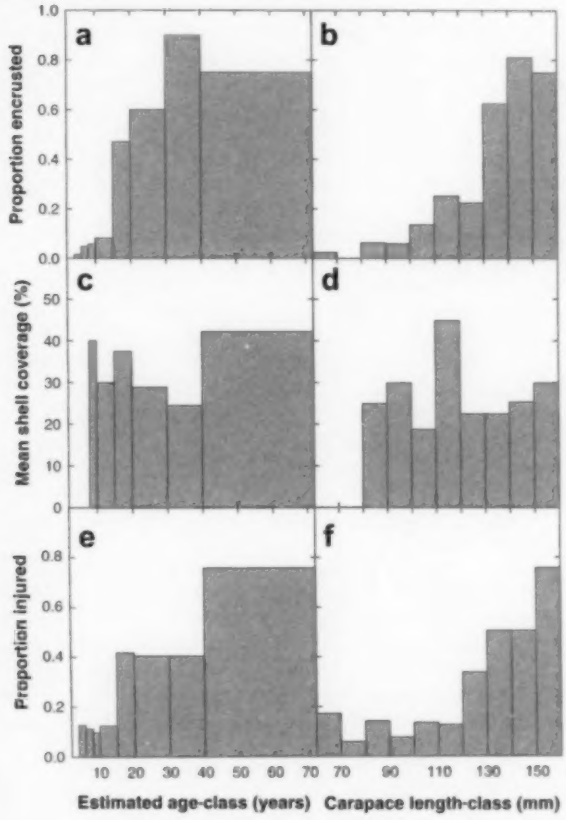
$$T = T_{S_{\infty/2}}\left(\frac{S_\infty}{S} - 1\right)^{1/-g}$$

where T is estimated age (years), S is either OCL (millimetres) or live mass (kilograms), S_∞ is the predicted average maximum size, g is the instantaneous rate of growth, and $T_{S_{\infty/2}}$ is the age at half the asymptotic size. Novel symbolism was chosen to avoid confusion with conventional models. Parameter estimates are shown in Fig. 4. Data smoothing had little effect on these.

The models indicate a slower average growth rate for adult females than for males, with generalized growth curves for OCL beginning to diverge at 3–4 years of age. The growth curves intercept MLS at 7.7 years for males and at 9.3 years for females. When calculated directly from the sample age distribution, the average age of both males and females in the 80.0–89.9 mm OCL class (within about one moult either side of the MLS threshold) was 7–8 years. The modal age in this size range for both sexes was 9 years. Average mass differ-

© 1999 NRC Canada

Fig. 6. Shell condition of sampled wild Yorkshire *H. gammarus* (males and females pooled): proportion of encrusted lobsters sampled in each estimated (*a*) age- and (*b*) size-class, average epibiont surface area coverage for encrusted lobsters within each estimated (*c*) age- and (*d*) size-class, and proportion of lobsters with old injuries including missing appendages or shell damage in each estimated (*e*) age- and (*f*) size-class.



ence between adult males and females of similar age became increasingly pronounced with advancing age (Fig. 5*b*) as male claw volume increased disproportionately.

Shell condition and injury

Old shell damage or appendage loss, some of it obviously inflicted by the chelae of other lobsters, was exhibited by 18% of sampled wild lobsters in each sex. As judged by the presence of epibiotic growth on the shell, 25% of individuals in each sex had not moulted recently. The relationship between estimated age and proportion of old-shelled lobsters was distinctly sigmoidal in appearance, with a sharp rise at about 15 years of age (Fig. 6*a*). The proportion of sampled lobsters in the predominant catchable size range (60–100 mm OCL) that had moulted recently was about 96% but gradually decreased with increasing size (Fig. 6*b*). Numbers of observations were insufficient to subclassify the data by sex. In old-shelled lobsters, there was no obvious relationship between surface area covered and age or size of the individual (Figs. 6*c* and 6*d*). Proportion of lobsters showing some form of old injury increased gradually with both age and size (Figs. 6*e* and 6*f*).

Discussion

Age–length and age – lipofuscin concentration relationships in the reference sample

The size-at-age and MTALC-at-age relationships for the Yorkshire reference lobsters are similar to those obtained previously for Orkney lobsters (Sheehy et al. 1996). A full statistical comparison including all landing sites will appear elsewhere. The poor age–length relationship in the present reference sample is representative of the full Bridlington tag–recapture data set (n = 621) (Bannister et al. 1994).

Although a highly significant relationship ($p < 0.0001$) between age and MTALC existed, age still explained only 31% of the variance. The relatively narrow 95% CL for individual mean MTALC estimates (Fig. 2*a*) demonstrates the efficacy of the lipofuscin assay procedure itself; even the highest mean MTALC measured in any of the lobsters, 7.4% VF, had 95% CL of only ±0.5% VF. Error around the regression is largely inherent individual variation, but it is important to put these data in context. The MTALC range of the reference sample was only 6% of that present in the wild lobsters and, when viewed through such a small window, "noise" becomes a significant factor in the total variance.

Age prediction methodology

Although MTALC proved to be a significantly better ageing criterion than size, as expected from previous studies (e.g., Sheehy 1990; Sheehy et al. 1996), many of the lipofuscin-based age determinations for the wild lobsters depend heavily on the form and precision of extrapolation from the reference data. The 95% PL and bootstrap 95 percentiles for the linear regression (Fig. 3*a*) show that confidence in the older age estimates is strongly influenced by the small available age range and consequently low coefficient of determination of the reference data. This is inevitable for such a long-lived species. However, use of the more comprehensive data sets from the shorter-lived astacidean species to make inferences about the extrapolated portion of the age – lipofuscin concentration relationship in *H. gammarus* was supported by the observed uniformity of lipofuscin variance patterns between the three species, despite substantial habitat and life history differences.

Regarding linearity, many previous studies have shown strong linear relationships between age pigment concentration and chronological age, while others have shown curvilinear relationships (see Sheehy 1992). Studies on freshwater crayfish (Sheehy et al. 1990) and American lobsters, *Homarus americanus*, up to 27 months of age (Wahle et al. 1996) suggest a period of relatively slow lipofuscin accumulation in the very earliest part of life, but these age groups are not relevant to the present study. At the opposite extreme, apparent decline in age pigment accumulation rate at older ages has also been shown (Donato et al. 1979; Sheehy 1992; Sheehy et al. 1994, 1995). Three explanations have been proposed for this observation. Firstly, it could be a normal correlate of slowing metabolism in ageing neurones (Sheehy et al. 1995) or, secondly, an artefact of age-specific mortality in experiments that have, by necessity, sampled

© 1999 NRC Canada

laboratory populations at different ages rather than the same individuals over time (Donato et al. 1979). Thirdly, the decline may be an artefact of abnormal physiological depression due to prolonged rearing under unnatural laboratory conditions (Sheehy et al. 1994). This possibility was supported by observations on freshwater crayfish that, when grown under field conditions, *C. quadricarinatus* did not exhibit the same declining lipofuscin accumulation rate that the laboratory populations did (fig. 1*b* in Sheehy et al. 1994). This supports the view that linear extrapolation in the present study is reasonable, but the topic requires further investigation using short-lived models. The effect of a real natural decline in lipofuscin accumulation rate in the oldest lobsters on the present results would be that their true ages are greater, *M* values lower, and the upper part of the generalized growth curves flatter than we have estimated.

With respect to error around the prediction model, some inflation of lipofuscin concentration variability, at ages greater than those in the reference sample, is expected from previous studies (e.g., Figs. 3*b* and 3*c*). However, the mean-to-variance (signal-to-noise) ratio also increases so that the relative resolving power of the lipofuscin-based age index is highest at the oldest ages.

Longevity, physiological ageing, and *M*

The estimates of potential longevity obtained here are first approximations of what is likely to be found in nature. While the largest females sampled (150–169.9 mm OCL) were of size comparable with that of the largest female recorded historically (170 mm rostral carapace length, Wolff 1978), the largest males sampled, again 150–169.9 mm OCL, were substantially below the record of >250 mm rostral carapace length (Wolff 1978). Male potential life span therefore may well exceed the 31-year average estimated here, especially as lipofuscin provides no evidence of different rates of physiological ageing between the sexes.

While little is known about ageing generally in the Crustacea, previous studies have shown that *Homarus* lacks some conventional signs of neuromuscular senescence (Govind 1995), and the present findings confirm that it has a potential life span longer than virtually all other arthropods (Comfort 1979). This relatively extreme longevity can be explained by both stochastic and adaptive theories of ageing. Senescence by accumulation of randomly damaged cellular components, as manifested by the lipofuscin deposition in the metabolizing postmitotic tissues of this large, sedentary, cool-water decapod, occurs about 20 times more slowly than in the much shorter-lived tropical *C. quadricarinatus*, for example (Fig. 3*c*). In an evolutionary context, once past the vulnerable larval stage, relatively low *M* is achieved, not by the acquisition of defensive envenomating organs, as in long-lived Anthozoa, for example, but rather by highly cryptic juvenile behaviour and heavy armouring of a relatively large body in adults. This would reduce natural selection pressure for rapid maturation, while a strong positive correlation between adult body size and reproductive performance (including egg size and larval viability) in both sexes (Attard and Hudon 1987; Cobb 1995; Estrella and Cadrin 1995; Waddy et al. 1995) could select for lengthened life span.

Although physiological age rather than body size may well be the primary correlate of maturation and variation in fecundity (e.g., Crocos and Coman 1997), defining age–fecundity relationships for large crustaceans has understandably proved difficult in the past. In the present Yorkshire lobster sample, there was a marked increase in the proportion of gravid females beyond a chronological age of about 12 years (Fig. 4*a*) and this is close to the age (15 years) above which there was also a sharp decrease in the proportion of moulters (Fig. 6*a*). An abrupt change in moulting patterns is known to be associated with sexual maturity in *H. americanus* (Ennis et al. 1986), so that these observations may indicate the approximate age of mass maturation for Yorkshire lobsters. Whether the absence of eggs on the three oldest females in the sample indicates a postreproductive senescent period in the lobster's life history, relevant to estimates of lifetime reproductive potential, requires further investigation. Both eyes of the oldest female were morphologically degenerate, raising the interesting possibility of an arthropod analogue to the age-related deterioration that occurs in mammalian eyes.

The longevity data permit the first measurements of *M* to be made for *H. gammarus*, and the values so obtained (females: 0.08, 8%/year; males: 0.15, 14%/year) indicate that the arbitrary assumptions made in previous stock assessments (0.1–0.2, Bannister 1986) were not unreasonable. Sex difference in *M* could arise from aggressive male territorial interaction or lower exposure of females to danger while sheltering when gravid.

Offshore refugia

Not all large lobsters are old; some grow rapidly. For example, the largest lobster sampled, a male of 166 mm OCL weighing 4.09 kg, from Cornwall, was estimated to be only about 11 years old. This shows that the commonly held view that gigantism equates with old age is an oversimplification, and it may be one explanation for the widely reported persistence of some large lobsters despite high exploitation rates and increased size-specific catchability (Addison 1997). Likewise, not all old individuals are large (the estimated 72-year-old female weighed only 2.07 kg). However, many of them are, and when the lobster's natural longevity is viewed as a life history optimization strategy to enhance reproductive success, this emphasizes the demographic importance of the old lobsters now being caught off the Yorkshire coast in an area where substrate shelter includes wartime wrecks. This is especially so if, as metapopulation analysis of *H. americanus* suggests, heavily fished inshore stocks could depend critically on larval subsidy from offshore (Fogarty 1998). It is clear that if fished out, these refugia could take many years to replace, and this may explain why some stocks have never recovered from heavy exploitation despite significant effort reduction (e.g., Watt and Arthur 1996).

Growth variation, recruitment, and selective fishing

Figures 4*a* and 5*a* show the size-stratified experimental sample and are not weighted by the total size distribution of landings. However, the age distribution within each size-class should be a good representation of that in the wild population. In this context, the narrow size range and low slope of the reference age–length relationship (Figs. 2*b* and 4*a*) (Bannister et al. 1994) are readily interpreted as a reflection of the wide age range, size-selective capture, and short life

© 1999 NRC Canada

expectancy of incoming size recruits rather than the true average growth trajectory.

Major features of the estimated age–length data are the pronounced individual variation in growth and differences in the pattern of that variation across the age range. Several lines of evidence support the conclusion that the variability is not primarily an artefact of age prediction errors: (*i*) the known-age material also shows high variability, (*ii*) Hepper's (1967, 1978) moult data predict very wide age–length variation, (*iii*) the lipofuscin back-calculated age range of the reference sample, 2.9–9.6 years, compares well with the actual age range of 4.3–8.4 years, (*iv*) in a recent study on *P. leniusculus* (Belchier et al. 1998), the difference between the actual age–length data and the lipofuscin back-calculated data was similarly small, and (*v*) wide individual variation in growth is typical for the Crustacea (e.g., Sheehy 1990; Belchier et al. 1998). The sex difference in growth variation encountered in the present study (r^2 in Figs. 4*a* and 5*a*) may reflect the more frequent aggressive encounters of males, which can induce strongly differential growth rates during artificial rearing (Cobb 1995).

The left-hand part of Fig. 4*a* shows the wide spread of ages associated with recruitment to the legal size range. Significant numbers of lobsters from at least seven year-classes (~5–11 years old) must recruit to the fishery at 85 mm OCL. Furthermore, the slowest growth extremes suggested by the lipofuscin-estimated age–lengths and Hepper's (1967, 1978) moult data indicate that all age-classes between about 3 and 15–20 years are probably only partially recruited to the fishery. Recruitment therefore extends well beyond the age of 8 years observed during stock enhancement studies (Bannister et al. 1994), where the cessation of monitoring after this time precluded the recapture of substantially older animals. The finding that the inshore sample contained few lobsters estimated to be older than 20 years (Fig. 4*a*, arrow 3), the point beyond which all age groups are expected to be fully exposed to fishing, suggests heavy exploitation of new recruits. The partial recruitment pattern therefore has several important implications as follows. (*i*) The present MLS provides protection for a substantial age range of lobsters even in the more heavily fished inshore waters. The plasticity of its growth format undoubtedly helps the lobster to survive under heavy fishing pressure, although additional studies are needed to define the position of reproducing females in this picture. (*ii*) It also indicates how significant changes in stocks can occur without being reflected in size distributions. Effects of variations in year-class strength will be damped by growth variation, tending to produce stable numbers of size recruits to the fishery over long time periods, but year-class failures may not be detected. (*iii*) Because recruiting size-classes are a cluster of many year-classes combined, attempts to develop recruitment indices based on early benthic phase lobsters will be complicated by the need to integrate data from several years of settlement. However, the cluster of 12- to 13-year-old lobsters in the present data (Fig. 4*a*, arrow 2), probably signifying a previous year-class strength fluctuation, suggests that MTALC–frequency analysis (Sheehy et al. 1998) may offer an alternative solution to the problem of establishing recruitment relationships. More detailed results on this topic will be published elsewhere.

Length-at-age variation is not uniform across the age range, being very much higher in younger lobsters than in older ones. Ricker (1969) concluded that this growth compensatory pattern, when observed in fish, was more likely to be a consequence of the exponential length–mass relationship than of selective mortality. However, another feature (arrow 1) of the scatter pattern in Fig. 4*a* is potentially significant in this regard. There appears to be a paucity of the largest individuals in all partially and fully recruited age groups, relative to the fastest growth extreme suggested by Hepper's (1967, 1978) moult data and a few fast-growing wild Yorkshire males (also apparent in Fig. 5*a*), a known-age male, and the single Cornwall male, all <12 years old, which are conspicuous outliers in a space otherwise devoid of lobsters that are relatively large for their age. One interpretation of this finding is that it is a natural growth phenomenon, perhaps resulting from the particular patterns of dominance hierarchy, shelter availability, and (or) population density that are present. Alternatively, the pattern could represent a fishing impact arising from a combination of size-selective harvesting and prolonged partial recruitment due to individual growth variation. The present results suggest that the fastest growing recruits, probably the dominant, more frequently moulting males controlling access to food, are potentially vulnerable to fishing for 7 years, or more, longer than the slower-growing individuals of the same cohort. Because the greater the within-cohort variation in size, the smaller the degree of size-selective mortality needed to produce a shift in mean length (Ricker 1969), the probability of a selective impact on these lobsters may be relatively high but not necessarily flagged by a high total mortality rate, many age groups persisting in the population. Size-selective fishing would represent an opposing force to any natural selection for large-bodied, high-quality, high-quantity reproducers. The surprisingly old ages of some undersized lobsters in the Yorkshire fishery may represent the selection for individuals that never exceed the legal size, as discussed by Jamieson et al. (1998) for Dungeness crab, *Cancer magister*.

Finally, the age–length and moult data do not support an assumption underlying the use of LCA, which is that differences in length can be related validly to differences in time/age (Jones 1974), even at sizes well below the asymptote. Variation in age-at-size, while extreme for larger sizes, is already wide by the time lobsters recruit to the fishery. Individuals clearly grow by unique and often asynchronous moulting pathways. The age–length relationships contained within the shaded area of Fig. 4*a* (males: $r^2 = 0.13$; females: $r^2 = 0.27$) suggest that the majority of size variation in lobster landings is not explained by age and therefore that the descending slope of the 5 mm OCL – frequency distributions used in LCA is primarily a reflection of individual growth variation rather than mortality.

Generalized growth

The von Bertalanffy growth model was used previously to describe the growth of lobster from tag–recapture data (Hepper 1978) for use in stock assessments (Bannister et al. 1983; Bannister 1986). However, the model is not always optimal for long-lived species (Cobb and Caddy 1989) and it does not adequately describe the present data owing to a poor fit for the older ages, where average size appears to in-

© 1999 NRC Canada

crease at a slow but constant rate. This apparent lack of an asymptote and the poor correlation between size or age and the amount of carapace encrustation (Figs. 6*c* and 6*d*), which should broadly reflect time elapsed since the previous moult, are consistent with an intermoult period that does not become increasingly long with advancing age, but rather is limited to a few years by the finite durability of the exoskeleton. In contrast, the new logistic model provided a good fit to the observed trends in both OCL and mass-at-age, for both sexes, across the whole range of ages (Figs. 4*b* and 5*b*). The model describes a trajectory that is slower in young lobsters than previously estimated (Fig. 4*c*). The resulting estimates of maximum size (216 mm OCL for males and 202 mm OCL for females) are similar to those quoted previously for United Kingdom fishing areas as a whole (210 mm OCL for males and 200 mm OCL for females, Bannister 1986), although the present female estimate differs somewhat from that previously quoted for Yorkshire (209 mm OCL for males and 169 mm OCL for females, Bannister et al. 1983). The present maxima still need to be treated with appropriate reservation because they lie well beyond the extensive age span of the data.

Some differences in estimated growth rate and maximum size are to be expected between the present results and previous ones based on Ford–Walford plots fitted to tag–recapture data. The latter may be biased in several ways as follows. (*i*) Increment data were difficult to obtain for lobsters much below 60–70 mm OCL, or much above 110 mm OCL (this draws attention to a major advantage of the lipofuscin method, which can provide growth information from the upper extremes of the size distribution after searching for the appropriate-sized animals in landings). (*ii*) Growth based on anniversary tag–recapture studies represents change in size averaged across the different ages present in a size-class, whereas a normal growth curve describes the average change in size across the individuals of one age. This difference was reduced in the present study by fitting the growth curve to the average age-at-size rather than the average size-at-age. (*iii*) In the case of Hepper (1978), data for captive male lobsters with intermoult periods of 2 or 3 years (25% of observations in the predominant catchable size range) were considered an artefact of artificial rearing and excluded from the analysis of MF because they were inconsistent with tag recaptures that showed only 0–17% of lobsters not moulting annually. This would have biased growth rate estimates upward if the inconsistency were due, instead, to a problem in the tag–recapture data. An assumption underlying estimates of average annual growth increment and MF from baited-trap studies, that lobsters that have moulted in the past year are sampled in the same proportions to their natural abundance as are lobsters that have not moulted, is questionable. The moult cycle is known to have a significant effect on catchability in the Yorkshire fishery. Moulting extends from February to October with a mid-summer (July) maximum characterized by low catch rates. This is immediately followed by a peak in catch per unit effort in August and September (Hepper 1978) that is consistent with the increased feeding activity expected in the postmoult recovery period (Cobb 1995). Direct use of age–length data for obtaining the growth curve circumvents these problems and demonstrates another advantage of the lipofuscin method.

Conclusions

The age–length analysis undertaken here is the first detailed description of growth based on chronological age for wild-caught clawed lobsters, and the discussion illustrates the insights that it gives into the population dynamics of lobsters off the Yorkshire coast. Conventional size-based methods for stock modelling have many shortcomings (Addison 1986), but their use has been unavoidable because age-based approaches that have been taken for granted in finfish research have long been lacking for crustaceans. The finding that the growth of Yorkshire lobsters is generally slower and much more variable than expected has implications for the results of previous length-based assessments using tag–recapture growth estimates, and this aspect is being investigated. The results illustrate the extremely protracted way that year-classes recruit to the fishery, and the resulting damping of year-class signals is probably a major cause of the stability in *Homarus* size distributions. The data have identified the likely significance to the population of the large and generally old lobsters from an offshore refuge. They also give rise to the question of whether the lobster population has experienced a selective fishing impact. Finally, as will be described in later papers, the application of the present age–length key to landings data will permit the calculation of age-specific mortality rates and an analysis of year-class strength and the causes of its variation. Clearly, the present paper describes the findings from only one sample from one coastal district. Further work to determine the full implication of the results for stock assessments and similar analyses on other United Kingdom lobster populations is needed, as well as further basic research on lipofuscin itself.

Acknowledgements

This project was funded by an MAFF Chief Scientists Group Award (CSG2596/MF0215). Centre for Environment, Fisheries and Aquaculture Science staff including Dr. M. Bell, Mr. S. Lovewell, and Dr. J. Addison at Lowestoft and Ms. H. Watkins, Ms. S. Ricketts, Mr. R. Arthur, and Mr. M. Smith at Conwy, and Dr. M. Becker, Oxford University, provided valuable assistance with various aspects of the study.

References

Addison, J.T. 1986. Density-dependent mortality and the relationship between size composition and fishing effort in lobster populations. Can. J. Fish. Aquat. Sci. **43**: 2360–2367.

Addison, J.T. 1997. Lobster stock assessment: report from a workshop. I. Mar. Freshwater Res. **48**: 941–944.

Attard, J., and Hudon, C. 1987. Embryonic development and energetic investment in egg production in relation to size of female lobster (*Homarus americanus*). Can. J. Fish. Aquat. Sci. **44**: 1157–1164.

Bannister, R.C.A. 1986. Assessment and population dynamics of commercially exploited shellfish in England and Wales. Can. Spec. Publ. Fish. Aquat. Sci. No. 92. pp. 182–194.

Bannister, R.C.A., Addison, J.T., Lovewell, S.R., and Howard, A.E. 1983. Results of a minimum size assessment for the fisheries for lobster, *Homarus gammarus*, in England and Wales. Int. Counc. Explor. Sea C.M. 1983/K:4.

© 1999 NRC Canada

Bannister, R.C.A., Addison, J.T., and Lovewell, S.R. 1994. Growth, movement, recapture rate and survival of hatchery-reared lobsters (*Homarus gammarus* (Linnaeus, 1758)) released into the wild on the English east coast. Crustaceana, **67**: 156–172.

Belchier, M., Edsman, L., Sheehy, M.R.J., and Shelton, P.M.J. 1998. Estimating age and growth of long-lived temperate freshwater crayfish using lipofuscin. Freshwater Biol. **39**: 439–446.

Cobb, J.S. 1995. Interface of ecology, behaviour and fisheries. *In* Biology of the lobster, *Homarus americanus*. *Edited by* J.R. Factor. Academic Press, New York. pp. 139–151.

Cobb, J.S., and Caddy, J.F. 1989. The population biology of decapods. *In* Marine invertebrate fisheries: their assessment and management. *Edited by* J.F. Caddy. John Wiley & Sons, New York. pp. 327–374.

Comfort, A. 1979. The biology of senescence. Churchill Livingstone, London, U.K.

Crocos, P.J., and Coman, G.J. 1997. Seasonal and age variability in the reproductive performance of *Penaeus semisulcatus* broodstock. Optimizing broodstock selection. Aquaculture, **155**: 55–67.

Donato, H., Hoselton, M.A., and Sohal, R.S. 1979. Lipofuscin accumulation: effects of individual variation and selective mortality on population averages. J. Gerontol. **14**: 141–147.

Ennis, G.P. 1977. Determination of shell condition in lobsters (*Homarus americanus*) by means of external macroscopic examination. Proc. Natl. Shellfish. Assoc. **67**: 67–70.

Ennis, G.P. 1979. Estimates of abundance and recruitment to the standing stock for a Newfoundland population of the lobster, *Homarus americanus*, with a method of estimating its natural mortality. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **175**: 225–228.

Ennis, G.P., Collins, P.W., Dawe, G., and Squires, W.R. 1986. Fisheries and population biology of lobsters (*Homarus americanus*) at Arnold's Cove, Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. No. 1438.

Estrella, B.T., and Cadrin, S.X. 1995. Fecundity of the American lobster (*Homarus americanus*) in Massachusetts coastal waters. ICES Mar. Sci. Symp. **199**: 61–72.

Fogarty, M.J. 1995. Populations, fisheries and management. *In* Biology of the lobster, *Homarus americanus*. *Edited by* J.R. Factor. Academic Press, New York. pp. 111–137.

Fogarty, M.J. 1998. Implications of migration and larval interchange in American lobster (*Homarus americanus*) stocks: spatial structure and resilience. *In* North Pacific Symposium on Invertebrate Stock Assessment and Management. *Edited by* G.S. Jamieson and A. Campbell. Can. Spec. Publ. Fish. Aquat. Sci. No. 125. pp. 273–283.

Govind, C.K. 1995. Muscles and their innervation. *In* Biology of the lobster, *Homarus americanus*. *Edited by* J.R. Factor. Academic Press, New York. pp. 291–312.

Hancock, D.A., and Edwards, E. 1967. Estimation of annual growth in the edible crab (*Cancer pagurus* L.). J. Cons. Cons. Int. Explor. Mer, **31**: 246–264.

Hepper, B.T. 1967. On the growth at moulting of lobsters (*Homarus vulgaris*) in Cornwall and Yorkshire. J. Mar. Biol. Assoc. U.K. **47**: 629–643.

Hepper, B.T. 1978. Population dynamics of the lobster, *Homarus gammarus* (L), off the coasts of England. Fish. Res. Tech. Rep. MAFF Dir. Fish. Res. Lowestoft No. 41.

Hoenig, J.M. 1983. Empirical use of longevity data to estimate mortality rates. Fish. Bull. U.S. **81**: 898–903.

Jamieson, G.S., Phillips, A., and Smith, B.D. 1998. Implications of selective harvests on Dungeness crab (*Cancer magister*) fisheries. *In* North Pacific Symposium on Invertebrate Stock Assessment and Management. *Edited by* G.S. Jamieson and A. Campbell. Can. Spec. Publ. Fish. Aquat. Sci. No. 125. pp. 309–321.

Jones, R. 1974. Assessing the long term effects of changes in fishing effort and mesh size from length composition data. Int. Counc. Explor. Sea C.M. 1974/F:33.

Krouse, J.S. 1989. Performance and selectivity of trap fisheries for crustaceans. *In* Marine invertebrate fisheries: their assessment and management. *Edited by* J.F. Caddy. John Wiley & Sons, New York. pp. 307–325.

Ricker, W.E. 1969. Effects of size-selective mortality and sampling bias on estimates of growth, mortality, production and yield. J. Fish. Res. Board Can. **26**: 479–541.

Sheehy, M.R.J. 1990. The potential of morphological lipofuscin age-pigment as an index of crustacean age. Mar. Biol. **107**: 439–442.

Sheehy, M.R.J. 1992. Lipofuscin age-pigment accumulation in the brains of ageing field- and laboratory-reared crayfish *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae). J. Exp. Mar. Biol. Ecol. **161**: 79–89.

Sheehy, M.R.J., Greenwood, J.G., and Fielder, D.R. 1994. More accurate chronological age determination of crustaceans from the field using the physiological age marker, lipofuscin. Mar. Biol. **121**: 237–245.

Sheehy, M.R.J., Greenwood, J.G., and Fielder, D.R. 1995. Lipofuscin as a record of rate of living in an aquatic poikilotherm. J. Gerontol. Ser. A. Biol. Sci. Med. Sci. **50**: B327–B336.

Sheehy, M.R.J., Shelton, P.M.J., Wickins, J.F., Belchier, M., and Gaten, E. 1996. Ageing the European lobster, *Homarus gammarus*, by the lipofuscin in its eyestalk ganglia. Mar. Ecol. Prog. Ser. **143**(1–3): 99–111.

Sheehy, M.R.J., Caputi, N., Chubb, C., and Belchier, M. 1998. Use of lipofuscin for resolving cohorts of western rock lobster (*Panulirus cygnus*). Can. J. Fish. Aquat. Sci. **55**: 925–936.

Sohal, R.S. 1981. Metabolic rate, ageing and lipofuscin accumulation. *In* Age pigments. *Edited by* R.S. Sohal. Elsevier/North Holland Biomedical Press, Oxford, U.K. pp. 303–316.

Sokal, R.R., and Rohlf, F.J. 1981. Biometry. W.H. Freeman and Company, San Francisco, Calif.

Waddy, S.L., Aiken, D.E., and De Kleijn, D.P.V. 1995. Control of growth and reproduction. *In* Biology of the lobster, *Homarus americanus*. *Edited by* J.R. Factor. Academic Press, New York. pp. 217–266.

Wahle, R.A., Tully, O., and O'Donovan, V. 1996. Lipofuscin as an indicator of age in crustaceans: analysis of the pigment in the American lobster, *Homarus americanus* (Milne-Edwards). Mar. Ecol. Prog. Ser. **138**: 17–123.

Watt, L., and Arthur, G. 1996. The improvement of the Shetland lobster fishery through stock enhancement. I. Fish. Dev. Note No. 2. North Atlantic Fisheries College, Shetland.

Wolff, T. 1978. Maximum size of lobsters (*Homarus*) (Decapoda, Nephropidae). Crustaceana, **34**: 1–14.

© 1999 NRC Canada

Spatial ontogeny of lentic age-0 rainbow trout, *Oncorhynchus mykiss*: whole-lake manipulations of population size structure

François Landry, John R. Post, and Eric A. Parkinson

Abstract: We assessed the influence of the presence of larger-bodied conspecifics on the spatial ontogeny of age-0 rainbow trout, *Oncorhynchus mykiss*. We created populations with three different size structures in experimental lakes: age-0 and larger-bodied conspecifics, age-0 only, and an initially age-0 only population into which we added larger-bodied conspecifics midexperiment. In the continuous presence of larger conspecifics, age-0 rainbow trout spatial distribution was skewed toward littoral and benthic habitats which had low abundances of their primary prey (zooplankton). The opposite pattern was observed in the experimental lake devoid of larger-bodied conspecifics where the largest proportion of the age-0 class was found in the open-water limnetic habitat where their prey was most abundant. The change in spatial distribution of age-0 rainbow trout in the lake with a midseason addition of larger-bodied conspecifics was consistent with a treatment effect. This addition effect was weakened by a significant ontogenetic change in spatial distribution from littoral to benthic to pelagic habitats as the age-0 rainbow trout grew, and the strength of the change was inversely related to the intensity of interactions with conspecifics. This study provides experimental evidence that the risk of interactions with larger-bodied conspecifics is the process that controls the ontogeny of habitat use by young fish in lakes.

Résumé : Nous avons évalué l'influence de la présence de congénères plus gros sur l'évolution de la distribution spatiale de truites arc-en-ciel d'âge 0, *Oncorhynchus mykiss*. Nous avons créé et placé dans des lacs expérimentaux des populations présentant trois structures de tailles différentes : des poissons d'âge 0 et des congénères de plus grande taille, des poissons d'âge 0 seulement et des poissons d'âge 0 seulement auxquels nous avons ajouté des poissons de plus grande taille au milieu de l'expérience. Dans le cas de la présence continue de congénères plus gros, l'aire de répartition des truites d'âge 0 a été déplacée vers les rives et les habitats benthiques où leurs principales proies (zooplancton) étaient peu abondantes. Un régime contraire a été observé dans le lac expérimental dépourvu de congénères plus gros et où la plus grande proportion des poissons d'âge 0 se trouvaient en eau libre dans l'habitat du limnion où leurs proies étaient les plus abondantes. La modification de la répartition spatiale des truites d'âge 0 dans le lac où des congénères plus gros ont été ajoutés à mi-saison correspondait à l'effet d'un traitement. Cet effet a cependant été affaibli suite à une évolution appréciable de la répartition des poissons qui sont passés des habitats riverains à des habitats benthiques et pélagiques à mesure de leur croissance, et l'importance du changement était inverse de l'intensité de l'interaction avec les congénères. L'étude démontre de façon expérimentale que les risques d'interactions avec des congénères plus gros sont à l'origine de l'évolution de l'utilisation des habitats par les jeunes poissons en milieu lacustre.

[Traduit par la Rédaction]

Introduction

Spatial segregation of species in freshwater fish assemblages is relatively common (Olson et al. 1988) and is also observed between age- and size-classes within species (Werner et al. 1983*b*; Magnan and FitzGerald 1984; Tonn et al. 1992; L'Abée-Lund et al. 1993; Langeland and L'Abée-Lund 1998). Habitat partitioning within species is usually a consequence of competition due to partial overlap in resource use among individuals of different size- or age-classes (Werner and Gilliam 1984; Venne and Magnan 1995). Empirical research on fishes has demonstrated that size-dependent competitive interactions within size- and age-structured populations determine recruitment and population dynamics (Hamrin and Persson 1986; Tonn et al. 1992; Post et al. 1999).

Interference competition involving aggressive interactions among individuals that preempts resources favours larger in-

Received November 17, 1998. Accepted July 6, 1999.
J14896

F. Landry[1] **and J.R. Post.**[2] Department of Biological Sciences, University of Calgary, 2500 University Drive N.W., Calgary, AB T2N 1N4, Canada.
E.A. Parkinson. British Columbia Ministry of Fisheries, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

[1]Present address: EVS Environment Consultants, 195 Pemberton Ave., North Vancouver, BC V7P 2R4, Canada.
[2]Author to whom all correspondence should be addressed. e-mail: jrpost@ucalgary.ca

© 1999 NRC Canada

dividuals, forcing smaller ones into less profitable habitats (Grant 1990). The extreme form of this interference behaviour is predation (Schoener 1983). Many studies have demonstrated that young fish modify their habitat use to reduce risk of interference or predation such as avoiding open water and selecting shallow, vegetated inshore areas (Werner et al. 1983*b*; Tabor and Wurtsbaugh 1991; Tonn et al. 1992). The use of habitats that provide refuge, or at least partial refuge, is common in freshwater fishes (Walters and Juanes 1993; Persson and Eklöv 1995; Eklöv and Persson 1996).

Most of the work on size-dependent interactions has focused on interspecific predation and its direct effects on prey species (Werner et al. 1983*a*; Tonn et al. 1992; L'Abée-Lund et al. 1993; Langeland and L'Abée-Lund 1998). However, intraspecific predation (cannibalism) may be equally important (Polis 1981; Foster et al. 1988), especially when life history stages differ considerably in size as in many species of fish. Therefore, inter- or intra-specific aggression, whether it results in predation or not, typically results in interference competition and differential habitat selection by smaller and younger individuals. The spatial and temporal distribution of small or young fish is often also associated with seasonal changes in resource levels leading to changes in habitat profitability (Werner et al. 1983*b*; Hamrin and Persson 1986). Therefore, small, young fish are faced with a trade-off between risk of aggressive interactions that could lead to mortality or of additional energetic costs and foraging profitability as they choose habitats (Werner et al. 1983*a*, 1983*b*; Gilliam and Fraser 1987; Walters and Juanes 1993).

Dominance hierarchies formed by size-dependent interactions occur in salmonid populations living in lotic systems (Li and Brocksen 1977; Grant 1990; Huntingford 1993). The interactions are a result of competition between individuals through aggressive behaviour to obtain stream positions that provide the greatest net energy gain (Grant and Noakes 1988). Stream salmonids have to compromise between limiting energy expenditure while swimming against a current and increasing exposure to drifting invertebrates and potential predation risk. Therefore, because lotic systems are structured physically and biologically in features such as current velocity, prey availability, and predation risk, dominance hierarchy develops to defend territory or optimal stream position (see review Huntingford 1993). These same salmonid species also live in lentic systems, where they do not experience the same physical and biological gradients as in lotic systems. It is not presently known whether salmonids living in lentic systems show the same size-structured spatial behaviour.

Most experiments dealing with size-dependent interactions among fish have been conducted at small scales such as aquaria, laboratory streams, enclosures, ponds, or sections of ponds as the experimental units. These conditions restrict a fish's behaviour due to the limited availability of alternative habitats. It is therefore critical to use whole-system experiments to study size-dependent interactions to incorporate both spatial and temporal natural variability in these interactions (Tonn et al. 1994; Persson et al. 1996). Conversely, most surveys of the spatial distribution of fish at a natural scale are not amenable to experiments to test ideas about the processes underlying observed patterns in size-dependent use of space.

The goal of our research is to assess the importance of body size dependent competitive or cannibalistic interactions to habitat use of age-0 rainbow trout, *Oncorhynchus mykiss*. Rainbow trout is a useful test species because a large background literature provides details of their natural history. They experience size-dependent cannibalism (Tabor and Wurtsbaugh 1991; Post et al. 1998), competitive interference (Li and Brocksen 1977; McCarthy et al. 1992), and exploitative competition (Post et al. 1999). Adult rainbow trout spawn in streams and juveniles migrate from streams into lakes at ages ranging from shortly after hatch to age 3 (Northcote 1969; Rosenau 1991), and therefore, several age- and size-classes cooccur in lakes. Previous studies have shown that adult rainbow trout use the epilimnion of lakes (Warner and Quinn 1995) and that age-0 rainbow trout tend to occupy the shallow littoral zone (Tabor and Wurtsbaugh 1991; Post et al. 1999). Post et al. (1999) demonstrated that age-0 and older rainbow trout overlap in use of space and food resources. The goal of our research is to understand the processes by which rainbow trout of different ages and sizes interact and how that is expressed in their use of space in lakes. Our approach involves varying the size structure in experimental populations and contrasting the use of spatial and food resources by two size-classes of rainbow trout. Specifically, we test the hypothesis that size-dependent interactions with larger conspecifics, spatial patterns in abundance of invertebrate prey, and spatial patterns in water temperature within lakes can explain the ontogeny of spatial distribution in age-0 rainbow trout. The approach adopted involves experimental manipulations of population size structure in three small lakes and observations of spatial patterns in habitat use and prey abundance over a single growing season.

Methods

Study area

The study was carried out in three lakes located on the southern interior plateau of British Columbia, 40 km southeast of Merritt (50°07′N, 120°47′W). The lakes are situated within a 0.56-km^2 area and consequently experience similar climatic and geological conditions. Their morphometries are similar (Table 1). These mesotrophic lakes range from 15 to 30 mg·L^{-1} in total phosphorus and from 300 to 500 mg·L^{-1} in total dissolved solids. Two of the lakes were fishless (due to winterkill) at the time of stocking, and the third had a very low density of age-1 rainbow trout with a known size structure and abundance. All lakes had been stocked with rainbow trout in previous years. Water temperatures in all habitats and in all lakes decreased from the beginning of the experiment at the end of July (22–23°C in the surface waters) to the end of September (15–16°C in the surface waters) (Landry 1997). Temperatures in the benthic habitats followed the same seasonal patterns but tended to be 1–3°C colder than surface waters.

All three lakes were stocked with rainbow trout reared from gametes collected from adults of a native population in Tunkwa Lake, B.C. We used fish with a wild ancestry as opposed to a hatchery ancestry because innate predator avoidance has been reduced through domestication in this species. The fish were reared at the Fraser Valley Trout Hatchery in Abbotsford, B.C. Two age-classes were used for the experiment: age-0 rainbow trout that had

© 1999 NRC Canada

Table 1. Morphometry and location of the experimental lakes in south-central British Columbia.

Physical parameter	Bluey Pothole 1 (age-0/age-1)	Bluey Pothole 2 (age-0 + age-1)	Kentucky Pothole (age-0)
Surface area (ha)	2.0	1.4	2.1
Perimeter (m)	710	508	601
Mean depth (m)	3.0	3.5	5.4
Maximum depth (m)	6.5	7.2	11.2
Habitat volume (1000 m^3)			
Littoral	6.78	5.01	4.47
Shallow epibenthic	6.16	3.13	2.88
Deep epibenthic	9.62	6.45	6.90
Limnetic	36.19	32.86	72.67
Mean Secchi disk depth (m)	4.4	4.1	4.1
Latitude	49°52′40″ N	49°52′50″ N	49°53′30″ N
Longitude	120°33′35″ W	120°33′35″ W	120°33′30″ W

hatched about 1 month prior to the experiment and age-1 rainbow trout that were 1-year-old fish. The fish were stocked in early July 1994, which corresponded to the timing of recruitment from streams into lakes for a proportion of the age-0 rainbow trout population in that region.

Experimental design

The main objective of this project was to examine the spatial distribution of age-0 rainbow trout among lakes with different size-structured populations. The experiment involved three stocked rainbow trout populations with different size structures. Bluey Pothole 1 was stocked with both age-0 and age-1 rainbow trout (age-0/age-1 treatment) where both age-classes were present throughout the entire experiment. Kentucky Pothole (age-0 treatment), received only age-0 rainbow trout so that inter-age-class interactions were absent. These two lakes acted as references for comparison with an experimentally perturbed system, Bluey Pothole 2. This treatment involved adding age-1 rainbow trout to the lake midway through the experiment, on August 19, that had previously contained only age-0 rainbow (age-0 + age-1 treatment).

All three lakes were stocked with age-0 fish at a target density of 10 800·ha^{-1}. Size at stocking was 30 ± 1.61 mm (SD) and 0.27 ± 0.07 g (SD). Bluey Pothole 1 was also stocked with age-1 rainbow trout measuring 157.9 ± 25.4 mm (SD) and weighing 44.8 ± 20.7 g (SD) at a target density of 750·ha^{-1}. Bluey Pothole 2 received age-1 rainbow trout that measured 209.4 ± 14.9 mm (SD) and weighed 101.2 ± 24.6 g (SD) and the target density was 750·ha^{-1}. Rainbow trout of these sizes have been shown to be facultative piscivores (Beauchamp 1990; Post et al. 1998). The target densities of age-0 and age-1 rainbow trout used in this experiment represent densities typical of monoculture rainbow trout lakes where stream spawning habitat is not limiting and angling exploitation rates are low (Post et al. 1999).

The study lasted 60 days and involved an intensive sampling of the spatial distribution of both age-classes of rainbow trout and their primary prey organisms, the zooplankton and benthos communities. The period allowed ontogenetic changes to occur and extended from the end of July 1994 to the end of September 1994. Each lake was sampled eight times during that period. The first sampling day was dropped from all analyses because age-0 rainbow trout had not yet grown to a size sufficient to be captured with gill nets in all lakes. Thus, for all analyses, we refer to sampling days 1–7. The period between sampling days varied between 5 and 10 days. The addition of yearlings in Bluey Pothole 2 (age-0 + age-1 treatment) occurred between sampling days 3 and 4.

We used a stratified random design to assess spatial and temporal patterns in rainbow trout abundance, invertebrate abundance, temperature, and oxygen concentration. The lake was initially arbitrarily divided into six habitats (upper littoral, lower littoral, shallow epibenthic, deep epibenthic, midshore limnetic, and limnetic) to cover all possible habitats used by the rainbow trout. The two littoral habitats were referred to as upper littoral (H1) and lower littoral (H2) (Fig. 1*a*) and were located at the 0.5- and 1.5-m depth contours, respectively. The midshore limnetic (H3) and the shallow epibenthic (H4) habitats were situated at the 2.5-m depth contour, with the midshore limnetic representing the top 1 m and the shallow epibenthic the bottom 1.5 m (Fig. 1*a*). At the 5.0-m depth contour, we sampled two habitats. The lower 1.5 m represented the deep epibenthic (H6) habitat and the limnetic (H5) was the upper 3.5 m. The upper and lower littoral, shallow epibenthic, and deep epibenthic were all situated within 1.5-m strata from the bottom of the lakes. However, for the analyses of fish distribution, we grouped both littoral (H1, H2) and both limnetic habitats (H3, H5) together and will therefore refer to four habitats (littoral, shallow epibenthic, deep epibenthic, and limnetic) because the upper littoral represented a low percentage of the total lake volume (Landry 1997) and the midshore limnetic had a low proportion (mean = 0.01) of the total catches. Fish were sampled in all six habitats, whereas the zooplankton community was sampled in five habitats (Fig. 1*b*). The macroinvertebrate community was sampled in two habitats (lower littoral and shallow epibenthic) (Fig. 1*c*), and temperature and oxygen concentration data were collected in all habitats between the day and the dusk fish sampling period.

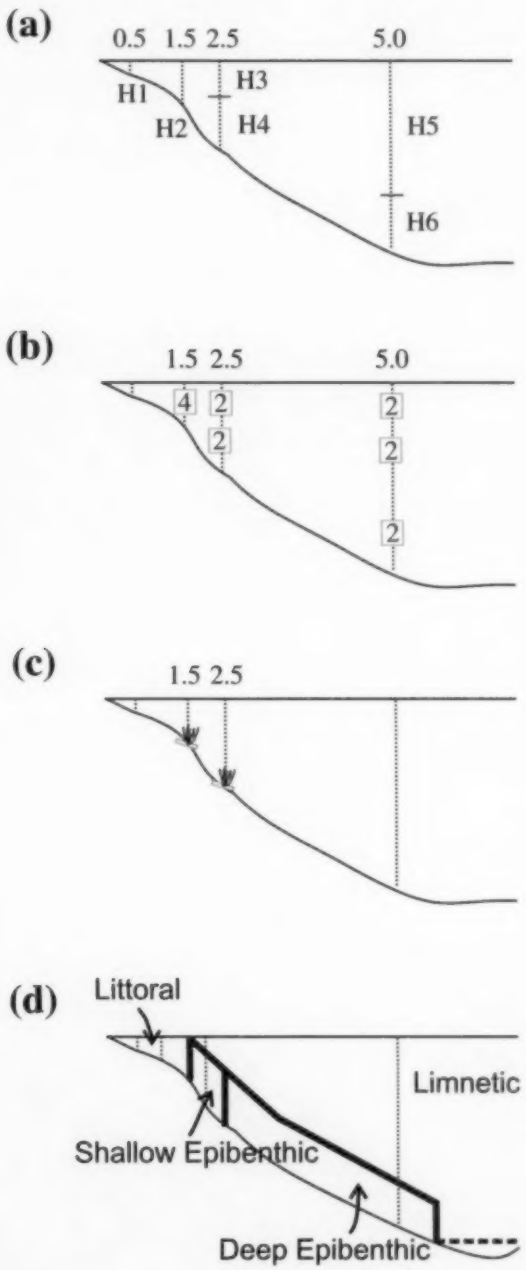
Sampling procedures

Fish

To sample age-0 fish, we used Lundgren multiple-mesh experimental gill nets, which are slow-sinking nets made with monofilament nylon. They are 11.4 m long and are divided into four equal panels of 13-, 16-, 20-, and 25-mm stretched mesh. We used gill nets of four heights, 0.5, 1.5, 2.5, and 5.0 m, to sample all the habitats of the lake. We delineated the 2.5- and 5.0-m nets with a horizontal string through the entire length of the net at 1.5 m from the bottom that separated the epibenthic habitats (H4, H6) from the limnetic habitats (H3, H5) (Fig. 1*a*). We established 10 fixed sites uniformly along each of the 0.5- and 1.5-m depth contours for the two littoral habitats and six sites for each of the 2.5- and 5.0-m depth contours. On each sampling day, one to five sites were selected randomly from the 10 sites for each of the littoral habitats (H1, H2), and one to three sites were selected randomly from the six sites for each of the other two depths. The precise number of nets set in each habitat was chosen to obtain a sample of about 200 fish. All nets were set for 1 h in the afternoon between about 12:30

© 1999 NRC Canada

Fig. 1. (*a*) Location of the six sampling habitats and the four gillnet sampling depths (vertical dotted lines). (*b*) Location of the zooplankton samples and number of samples per habitat. The first depth from the surface is at 1 m, the next at 2 m, and the deepest at 4 m. (*c*) Depth of the macroinvertebrate samples. (*d*) Division of the lake for each habitat volume calculation. The thick broken line represents the 6-m depth contour.



and 14:30 and for 1 h at dusk. Nets set at dusk were set 0.5 h before sunset and were retrieved 0.5 h after sunset.

Larger-mesh gill nets were used to sample age-1 fish during the day only. They were sampled on every second sampling day in the age-0/age-1 treatment and on all 4 days following the addition of age-1 fish in the experimental treatment lake (age-0 + age-1 treatment). age-1 rainbow trout were sampled in three habitats: 1.5 m (littoral), 2.5 m (shallow epibenthic), and 5.0 m (limnetic and deep epibenthic). Nets of two different heights (2.3 and 4.9 m) were used to sample these habitats. These nets were gangs of seven panels, each with a different mesh size (ranging from 25- to 89-mm stretched mesh) and measuring 14.6 m in length for a total length of 102.2 m. The fish caught in all habitats were counted and the age-class was recorded.

Zooplankton

The zooplankton community was sampled with a Schindler trap in five of the six habitats on each sampling day between the day and dusk gillnetting periods (Fig. 1*b*). The upper littoral (H1) habitat was not sampled because it was too shallow. The samples were collected where nets were set to collect fish. On each sampling day, four lower littoral (H2) habitat sites were sampled at 1 m depth and these samples were combined. Two samples were collected at 2 m depth for the shallow epibenthic (H4) habitat and two at 4 m depth for the deep epibenthic (H6) habitat (Fig. 1*b*). Six samples were collected and combined for the limnetic habitat, including two samples from 1 m depth in the midshore limnetic (H3) sites and four samples in the limnetic (H5) (two at 1 m depth and two at 2 m depth). The zooplankton samples were preserved in 70% ethanol. In the laboratory, a subsample of not less than 300 individuals were identified to genus and measured to the nearest millimetre using a dissecting microscope at 25×. Biomass of individuals was obtained from length–weight regressions for each taxon. The total biomass of zooplankton was then calculated for each habitat, on each sampling day.

Macroinvertebrates

Macroinvertebrates were sampled four times during the study period with an artificial substrate sampler (Benoît et al. 1998). The samplers captured the larger and more mobile macroinvertebrates that make up the majority of macroinvertebrates eaten by rainbow trout (Benoît et al. 1998). All the lakes were sampled for benthic macroinvertebrates on sampling days 1, 3, 5, and 7. The artificial samplers were constructed from a 20 × 20 cm ceramic tile with 12 fronds of plastic "macrophytes" (measuring 9 and 18 cm in height) glued on the tiles. These macrophytes simulated the littoral flora of the lakes and were most similar to *Myriophyllum* sp., which was dominant in the lakes. The tiles were positioned by a snorkeling diver at two depths (Fig. 1*c*) that corresponded to the bottom of the lower littoral (H2) habitat at 1.5 m and the shallow epibenthic (H4) at 2.5 m. Five replicates were placed at each depth and the locations were randomly selected from 20 fixed sites at each depth. The tiles were covered and retrieved after a 4-day colonization period by a snorkeling diver using a Plexiglas box that prevented loss of organisms on the tile and artificial macrophytes. Once out of the water, the water in the box flowed out the bottom through a 500-μm mesh and the invertebrates were retained on the mesh in the box. The fronds and the surface of the tile were washed with a high-pressure water jet. Additional detail on the methodology and colonization dynamics of macroinvertebrates on the artificial substrate samplers is available in Benoît et al. (1998). The invertebrates were preserved in 70% ethanol. In the laboratory, they were sorted, identified to order or family, and counted with the use of a dissecting microscope. The invertebrates were blotted to remove superficial water and each taxon was weighed as a group to the nearest 0.1 mg.

© 1999 NRC Canada

Data analysis

Analysis of the spatial distribution of a population requires estimates of the relative proportions of the total population inhabiting various habitats. Commonly, catch-per-unit-effort (CPUE) measures are used to contrast distribution patterns among habitats, but this is inappropriate because the area or volume of habitats sampled varies among natural lakes. CPUE is proportional to density within a habitat, but it gives no indication of the proportion of the population using a particular habitat if the volume of habitats varies within and among lakes, which is certainly typical (Table 1). Therefore, our estimates of spatial distribution were based on CPUE from the gillnet catches multiplied by the volume of water in the habitat in which the nets were set. An index of the total population, hereafter referred to as "population index," was the sum of the CPUE multiplied by volume estimates across all habitats within each lake (Fig. 1*d*):

$$(1) \qquad P_{\text{index}} = \sum_{H_1}^{H_x} (\text{CPUE} \times \text{volume})$$

Spatial distribution is presented as the relative proportion of the population index that occurs in each of the four habitats within a lake ($\%P_{\text{index}}$ per habitat).

Volumes of each habitat were calculated from bathymetric maps by simply multiplying the surface area by mean depth. The surface area was determined by delineating each habitat by the depth contour at the midpoint between the center of adjacent habitats (i.e., 1.0 m delineates the division between H1 and H2; 2.0 m delineates the division between H2 and H3, H4) with the exception of the H5 and H6 habitats for which the 3.0-m depth contour was defined as the shallowest extent of these habitats.

The spatial distribution data set therefore comprised estimates of the proportion of the population index for each net set in each habitat for each of 7 sampling days. Many nets did not catch fish, so the data were not normally distributed and the variances were not homogeneous. We consequently used a two-step process to analyze spatial distribution over habitats, over time, and among lakes. First, we assessed the spatial distribution of age-0 rainbow trout among different treatment lakes and over time with a three-factor ANOVA by using only the nonzero values of the population indices in an unbalanced design with the number of replicates varying from one to 10 for each habitat. After a log transformation of the nonzero values, the data were normalized (Kolmogorov–Smirnov test, $p > 0.28$) and variances were homogeneous (Bartlett's test, $p > 0.08$). We conducted the ANOVA for only the dusk data because from the 388 nets set during the day for the whole study, 167 had no fish; hence, too many cells had no observations. Only 48 out of 346 nets set at dusk had no fish. We had to delete sampling day 1 from the 7 sampling days in the analysis because on one occasion, there was a cell with no observations (no fish captured).

The second part of this two-step process involved analyzing all estimates of proportion of total population index from each net catches, including the zero values, by examining the presence or absence of fish in nets across sites and treatments and lakes. The capture success of the nets was modeled using a logit model (Collett 1991). We analyzed capture success using a logistic regression on the success of a net (the net did or did not catch fish) with the LOGIT function from a supplementary module for SYSTAT (Steinberg and Colla 1991). We performed this regression to determine how factors including treatment, habitat, sampling day, and pre/post-addition influenced the capture success of a gill net. We first contrasted the capture success among the three lake treatments and then used independent analyses for each treatment for the three independent variables. Sampling day was the only continuous variable. To maintain consistency with the three-factor ANOVA, we omitted sampling day 1 from the analysis. In the analyses, there were a total of 150 nets for the age-0/age-1 treatment, 148 for the age-0 + age-1 treatment, and 48 for the age-0 lake. The low number of nets in the age-0 lake was due to its high catch rates.

Table 2. Three-factor ANOVA on population indices from dusk samples of age-0 rainbow trout with respect to treatment, sampling day, and habitat over 6 sampling days for four habitats.

Source	SS	df	MS	*F* ratio
Treatment	8.19	2	4.09	32.52***
Sampling day	0.73	5	0.15	1.17
Habitat	3.33	3	1.11	8.80***
Treatment × sampling day	1.37	10	0.14	1.09
Treatment × habitat	2.55	6	0.43	3.37**
Sampling day × habitat	0.70	15	0.05	0.37
Treatment × sampling day × habitat	3.67	30	0.12	0.97
Error	24.05	191	0.13	

Note: All cells in the analysis with population indices of zero were omitted. $^{**}p < 0.01$, $^{***}p < 0.001$.

We analyzed the spatial distribution of zooplankton using a single-factor ANOVA on the total zooplankton biomass per litre for each lake separately with habitat as the main effect using sampling days as replicates. To test the alternate hypothesis that the fish distribution correlates with zooplankton density, we calculated Spearman's coefficient of rank correlation (Sokal and Rohlf 1995) between the fish distribution at dusk and the zooplankton density in the four habitats. We calculated this coefficient for each sampling day in each treatment. To reject the null hypothesis that there is no correlation between the two sets of rankings with a sample size of four required a perfect correlation ($r_s = 1$). Therefore, we used this analysis only to qualitatively describe patterns, since the small sample size made it impossible to test the hypothesis of no correlation with power.

The spatial pattern of macroinvertebrates was analyzed similarly but with only two habitats. We performed a single-factor ANOVA on the total biomass per tile with habitat as the main effect for each lake using sampling days as replicates. Data for both zooplankton and macroinvertebrates analyses were log transformed to ensure normality and homogeneity of variances. A Kolmogorov–Smirnov test showed that the data for both zooplankton and macroinvertebrates were not significantly different from a normal distribution ($p > 0.05$) in all three treatments. Bartlett's test resulted in homogeneous variances ($p > 0.05$) for zooplankton and macroinvertebrate data in the three lakes. To facilitate presentation, descriptive statistics were back-transformed, resulting in asymmetric 95% confidence limits.

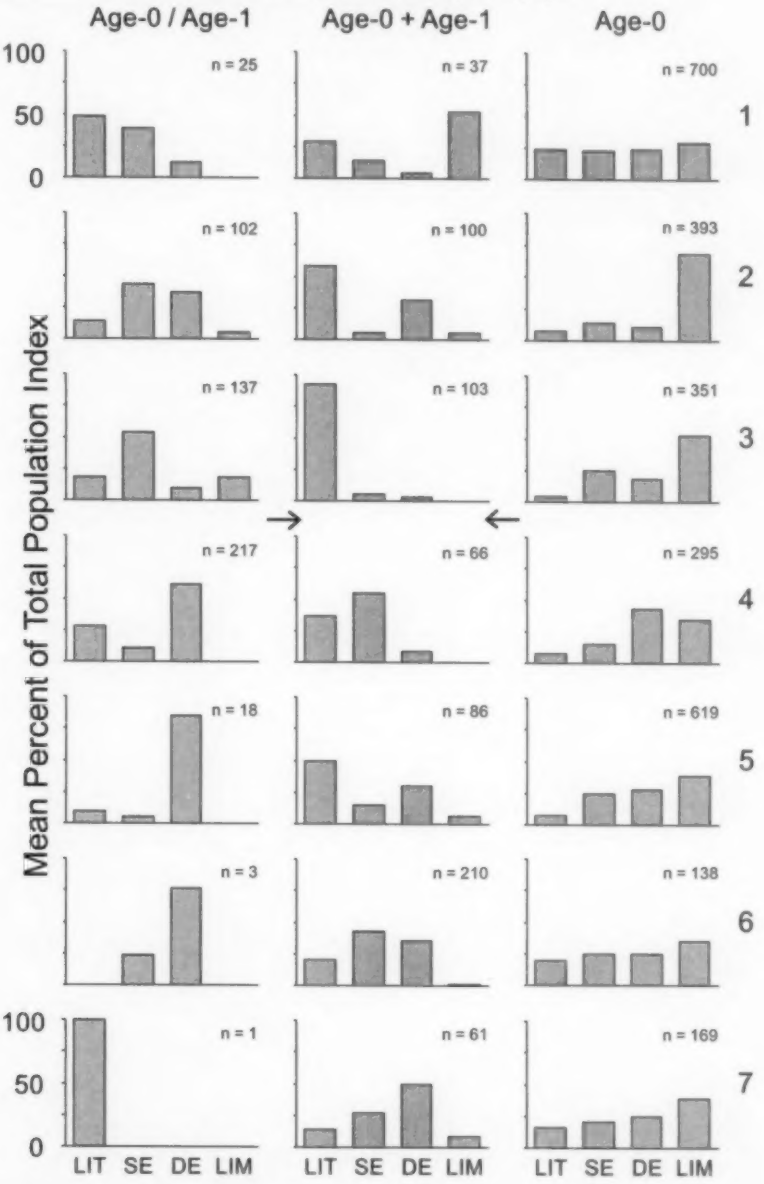
Results

Spatial distribution of age-0 and age-1 rainbow trout

The spatial distribution of age-0 rainbow trout varied day to dusk, over time, and among treatments (Figs. 2 and 3). A three-factor ANOVA showed significant treatment × habitat, treatment, and habitat effects on the spatial distribution of age-0 rainbow trout at dusk (Table 2). The two-way interaction implies that there are significant differences in the spatial distribution of age-0 rainbow trout among the three lakes. In the continuous presence of older conspecifics (age-0/age-1), the fish distributed themselves uniformly across the four habitats (Fig. 4), whereas in the age-0 + age-1 treatment, fish used the deep epibenthic habitat significantly

© 1999 NRC Canada

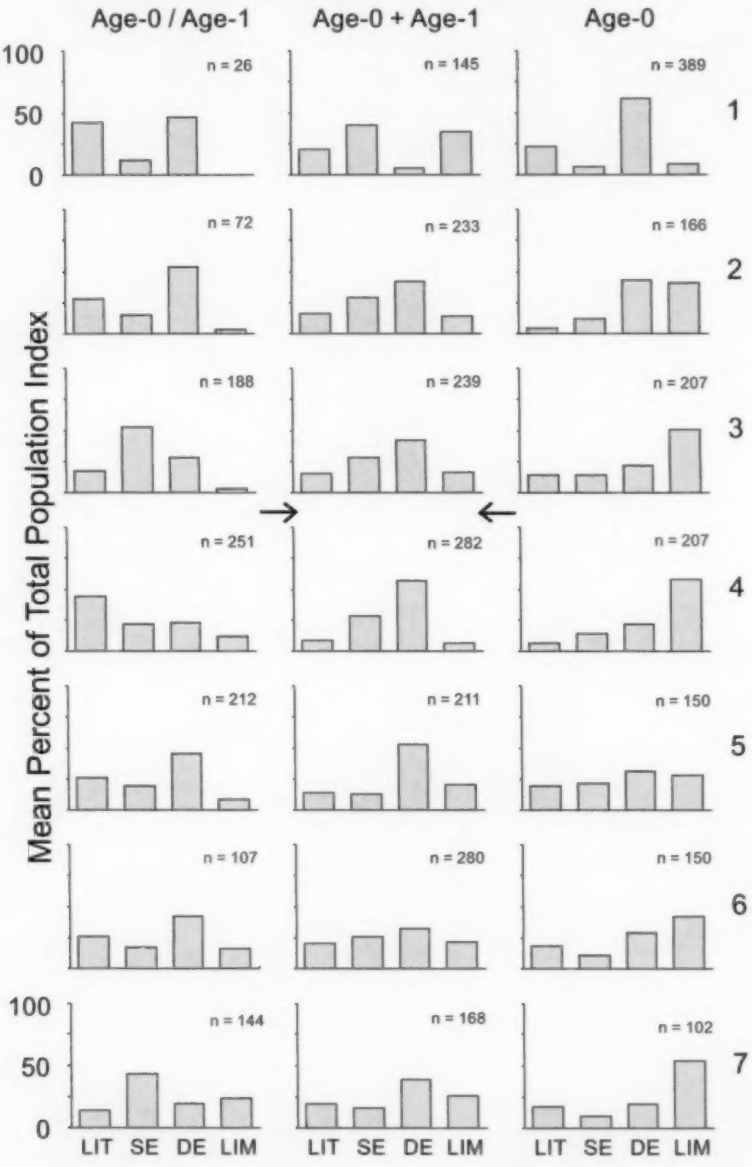
Fig. 2. Daytime distribution of age-0 rainbow trout for each treatment for the 7 sampling days from the end of July to the end of September. Data are means of replicate nets from each habitat presented as a percentage of the total population index. The arrows indicate the time of addition of age-1 rainbow trout to the experimental lake (age-0 + age-1). LIT, littoral; SE, shallow epibenthic; DE, deep epibenthic; LIM, limnetic. The number of fish sampled on each day is displayed.



more than the littoral and limnetic habitats. In the total absence of older conspecifics, the age-0 fish were significantly more abundant in the limnetic than in the littoral habitat. We were not able to test statistically the distributional patterns of age-0 fish during the day due to the large number of zero catches in the nets, but similar patterns to the dusk distribution were observed (Fig. 5). A representation of the mean percentages of the population indices for each habitat in each lake enabled us to observe differences among lakes. The mean percentages within a habitat clearly differed among the lakes for both day and dusk (Fig. 5). In the littoral zone, the habitat with the most complex structure, the lake without older conspecifics contained the lowest percentage of the population during both the day and dusk (Fig. 5), which may be considered as the least risky habitat. This pattern is the opposite for the limnetic habitat, likely the riskiest habitat. There was about a ninefold difference in the percentage of age-0 rainbow trout found in the limnetic

© 1999 NRC Canada

Fig. 3. Dusk distribution of age-0 rainbow trout for each treatment for the 7 sampling days from the end of July to the end of September. Data are means of replicate nets in each habitat presented as a percentage of the total population index. The arrows indicate the time of addition of the age-1 rainbow trout to the experimental lake (age-0 + age-1). LIT, littoral; SE, shallow epibenthic; DE, deep epibenthic; LIM, limnetic. The number of fish sampled on each day is displayed.



habitat between the age-0/age-1 and age-0 treatments during the day, whereas there was only a fourfold difference at dusk (Fig. 5). In the experimental lake in which age-1 rainbow trout were added midexperiment, the percentage was intermediate to that of the other two lakes in five out of eight instances. The high use of the littoral habitat in the experimental lake (age-0 + age-1) during the day is partly due to sampling day 3 where 90% of the total population index was obtained from one net in the littoral habitat for unknown reasons. The ratio of the total number of age-0 rainbow trout caught in the day to the total number of age-0 rainbow trout caught at dusk was with 0.50 with age-1 present throughout, 0.43 in the midseason addition lake, and 1.0 in the continuous absence of age-1 rainbow trout.

Complementary to the ANOVA of treatment effects, we performed four logistic regressions on the presence and absence of age-0 rainbow trout in nets for the dusk catches using treatment, habitat, sampling day, and pre/post-addition as

© 1999 NRC Canada

Fig. 4. Back-transformed means of population indices of age-0 rainbow trout from replicate nets with nonzero value catches for each habitat. Means are from dusk catches over 6 sampling days for all three treatments. Error bars represent upper and lower 95% confidence intervals. Different letters indicate a significant ($p < 0.05$) difference for a pairwise comparison on log population indices using Tukey's test. Data are based on sample sizes of 1000 fish for the age-0/age-1 treatment, 1558 fish for the age-0 + age-1 treatment, and 1371 fish for the age-0 treatment. All the *y*-axes have the same scales. LIT, littoral; SE, shallow epibenthic; DE, deep epibenthic; LIM, limnetic.

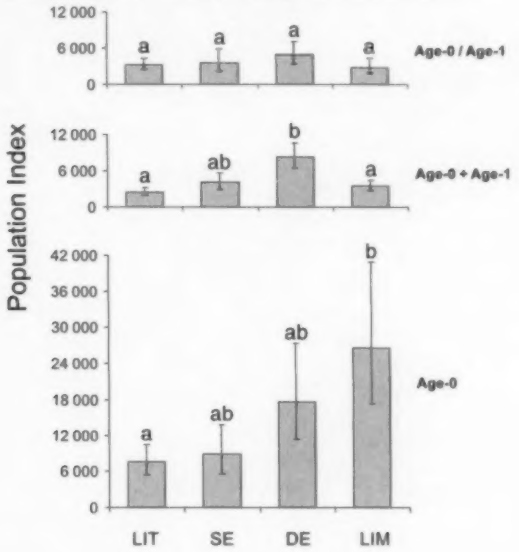
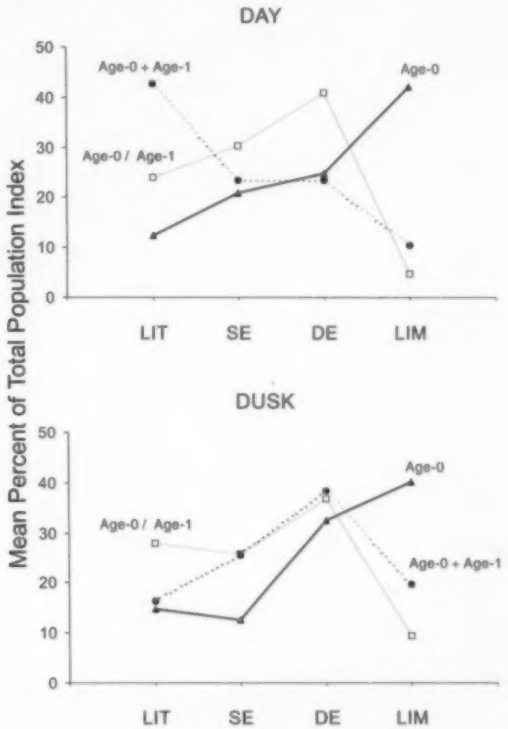


Fig. 5. Mean percentage of the total population index of age-0 rainbow trout for day and dusk catches over 7 sampling days for all four habitats. Data represent a mean of replicate nets in each habitat for 7 sampling days. Data include all nets with and without fish. Daytime data are based on sample sizes of 499 fish for the age-0/age-1 treatment, 663 fish for the age-0 + age-1 treatment, and 2665 fish for the age-0 treatment. Dusk data are based on sample sizes of 1000 fish for the age-0/age-1 treatment, 1558 fish for the age-0 + age-1 treatment, and 1371 fish for the age-0 treatment. LIT, littoral; SE, shallow epibenthic; DE, deep epibenthic; LIM, limnetic.



independent variables (Table 3). Standard errors cannot be calculated for the age-0 lake because all nets caught fish. The probabilities of success (presence–absence) are calculated and reported (Table 3). Gillnet success rate varied across lakes and among habitats within treatments. With treatment as a categorical variable, the age-0/age-1 lake was used as the reference group for the other two lakes. There were significantly different probabilities of capture success between the age-0/age-1 and age-0 + age-1 treatments. The lake with the continuous presence of age-1 rainbow trout had the lowest predicted probability of success, suggesting that not all nets caught fish, implying that fish are not using all habitats equally (Table 3). We examined differences across habitats within lakes and observed significant differences consistent with the spatial distribution results from the ANOVA analysis (Table 3). No regression was performed for the age-0 treatment due to catches in all nets. In the regressions for the other two treatments, we used the littoral habitat as the reference group. There were significant differences in capture success between the littoral and limnetic zones (Table 3). The low predicted probability of success in the limnetic habitat for the age-0/age-1 treatment infers a low use of that habitat in the continuous presence of age-1 rainbow trout. In the age-1 midseason addition lake, there is also a significantly lower success rate of nets set in the limnetic habitat than of those set in the littoral habitat.

The treatments of sampling day and pre/post-addition were analyzed independently. Sampling day was treated as a continuous variable, and we used the preaddition as the reference group for the pre/post-addition variable. There was a significant change in capture success across sampling dates in the continuous presence of larger conspecifics but not in the lake with the midexperiment addition of larger conspecifics (Table 3). There were no significant effects on capture success in the pre/post-addition models, yet the direction of the temporal pattern is consistent with an addition effect in the age-0/age-1 treatment.

The spatial distribution of age-1 rainbow trout in the two experimental lakes in which they were added at either at the beginning of the experiment or midexperiment did not differ (Fig. 6). In both lakes, about 80% of the age-1 fish inhabited the deep epibenthic and limnetic habitats (Fig. 6). Less than 20% of the age-1 rainbow trout inhabited the shallow epibenthic and midshore limnetic habitats at the 2.5-m depth contour, and less than 5% were found at the 1.5-m depth contour. Although the littoral zone shallower than 1.5 m was not assessed for age-1 rainbow trout, the exponential decline

© 1999 NRC Canada

Table 3. Individual logistic regressions of treatment, habitat, sampling day, and pre/post-addition effects on dusk gillnet catch success.

Treatment	Parameter	Estimate	SE	t ratio		P (success)		
				Treatment effects model				
Overall	α	1.686	0.243	6.928***		Age-0/age-1	0.84	
	β_1	1.147	0.459	2.499**		Age-0 + age-1	0.94	
	β_2	14.516	na	na		Age-0	1.00	
				Habitat effects model				
Age-0/age-1	α	2.872	0.593	4.839***				
	β_1	13.331	na	na				
	β_2	13.331	na	na				
	β_3	–2.760	0.681	–4.054***				
						Age-0/age-1	Age-0 + age-1	Age-0
Age-0 + age-1	α	3.970	1.009	3.933***	Littoral	0.95	0.98	1.00
	β_1	12.233	na	na	Shallow Epibenthic	1.00	1.00	1.00
	β_2	12.233	na	na	Deep Epibenthic	1.00	1.00	1.00
	β_3	–2.361	1.104	–2.138**	Limnetic	0.53	0.83	1.00
Age-0	α	na	na	na				
	β_1	na	na	na				
	β_2	na	na	na				
	β_3	na	na	na				
				Sampling day effects model				
					Sampling day	Age-0/age-1	Age-0 + age-1	Age-0
Age-0/age-1	α	0.292	0.655	0.446	2	0.72	0.90	1.00
	β_1	0.329	0.154	2.134*	3	0.78	0.92	1.00
Age-0 + age-1	α	1.604	1.030	1.557	4	0.83	0.94	1.00
	β_1	0.289	0.244	1.185	5	0.87	0.95	1.00
Age-0	α	na	na	na	6	0.91	0.97	1.00
	β_1	na	na	na	7	0.93	0.97	1.00
				Pre/post-addition effects model				
Age-0/age-1	α	0.969	0.354	2.738**				
	β_1	1.203	0.499	2.410		Age-0/age-1	Age-0 + age-1	Age-0
Age-0 + age-1	α	2.890	0.726	3.979***	Preaddition	0.72	0.95	1.00
	β_1	–0.081	0.860	–0.094	Postaddition	0.90	0.94	1.00
Age-0	α	na	na	na				
	β_1	na	na	na				

Note: Treatment, habitat, and pre/post-addition are used as independent categorical variables. The treatment model uses treatment age-0/age-1 as the reference group (α). Two dummy variables were created where the age-0 + age-1 treatment is represented by β_1 and the age-0 treatment by β_2. A regression model is presented for each treatment for the habitat, sampling day, and pre/post-addition logistic regressions. Each habitat model uses the littoral habitat as the reference group (α). Three dummy variables were created to represent the other three habitats. The shallow epibenthic habitat parameter is represented by β_1, the deep epibenthic by β_2, and the limnetic by β_3. Sampling day is used as an independent continuous variable. Each pre/post-addition model uses the preaddition category as the reference group (α). One dummy variable was created to represent the category postaddition (β_1). The predicted probability of success is calculated for each treatment separately, for each habitat in each lake manipulation, for each sampling day in each treatment, and for each addition period in each lake. Sampling day 1 was omitted from the analysis. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. na, not applicable.

in age-1 abundance with decreasing depth suggests that the shallower littoral zone is unlikely inhabited by age-1 rainbow trout.

Spatial distribution of invertebrate food

Data on the spatial distribution of zooplankton and macroinvertebrate taxa, which formed the diet of age-0 rainbow trout (Landry 1997), were collected to test the alternative hypothesis that age-0 rainbow trout distributed themselves in response to spatial variation in their prey community rather than in response to the presence of larger conspecifics. The littoral habitat had the lowest zooplankton density for most of the sampling days in all three lakes, which implies that it was not a profitable habitat for foraging (Landry 1997). The mean biomass differed approximately by an order of magnitude among lakes with a mean of 3261 mg·L^{-1} for the age-0/age-1 treatment, 2135 mg·L^{-1} for the age-0 + age-1 treatment, and 376 mg·L^{-1} for the age-0 treatment. A one-factor ANOVA for each treatment identified significant differences in prey densities across habitats (age-0/age-1: $F_{3,24} = 4.30$, $p < 0.05$; age-0 + age-1: $F_{3,24} = 5.76$, $p < 0.01$; age-0: $F_{3,24} = 7.89$, $p < 0.01$). In all three lakes, the littoral habitat had the lowest zooplankton biomass, and in all instances, it was significantly lower than the biomass in the limnetic habitat (Fig. 7).

The majority of Spearman's rank correlation coefficients between zooplankton biomass and age-0 rainbow trout distribution in the age-0 lake were positive (Table 4). For the age-0/age-1 treatment, the correlations were mostly negative (mean = –0.23). The experimental addition lake (age-0 + age-1) was intermediate with a mean of +0.37, but the 3 last sampling days had higher correlations.

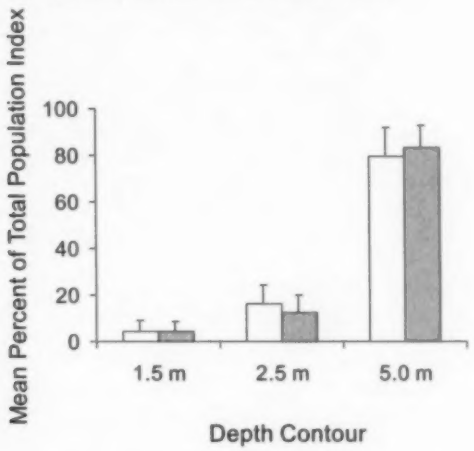
© 1999 NRC Canada

Table 4. Spearman's rank correlation for dusk rainbow trout distribution and zooplankton density ($\mu g \cdot L^{-1}$) during the study.

	Sampling day							
Treatment	1	2	3	4	5	6	7	Mean
Age-0/age-1	–0.6	–0.4	0.8	–0.8	–0.2	–0.8	0.4	–0.23
Age-0 + age-1	0	0.8	–0.4	–0.4	1	0.8	0.8	0.37
Age-0	–0.4	1	0.6	0.8	0.8	0.8	0.4	0.57

Note: All correlations are based on the ranks of the four habitats.

Fig. 6. Mean percentage of the total population index of the spatial distribution of age-1 rainbow trout. Open bars represent the age-0/age-1 treatment and shaded bars the age-0 + age-1 treatment. Data are means of replicate nets over 4 sampling days distributed throughout the experiment. The 1.5-m net represents the littoral habitat, the 2.5-m net the shallow epibenthic and midshore limnetic habitats, and the 5.0-m net the limnetic and the deep epibenthic habitats. Error bars are 1 SD.

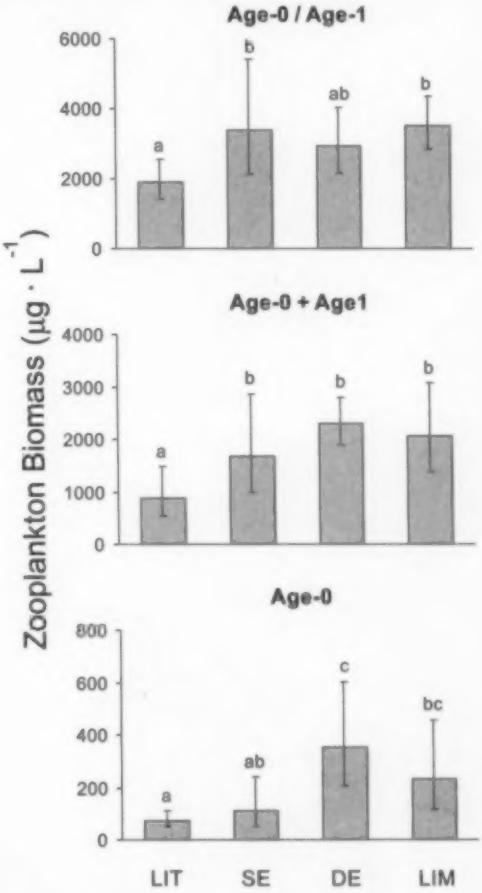


A one-factor ANOVA was conducted on the biomass of macroinvertebrates per tile between habitats for each lake, and there was a significant difference between the two habitats sampled in only the experimental lake (age-0/age-1: $F_{1,38} = 1.16$, $p > 0.05$; age-0 + age-1: $F_{1,38} = 9.77$, $p < 0.01$; age-0: $F_{1,38} = 2.04$, $p > 0.05$) (Fig. 8). The trend in the experimental addition treatment (age-0 + age-1) shows more age-0 rainbow trout in the shallow epibenthic habitat than in the littoral habitat, which is positively correlated with a significant difference in macroinvertebrate abundance between habitats (Fig. 8).

Discussion

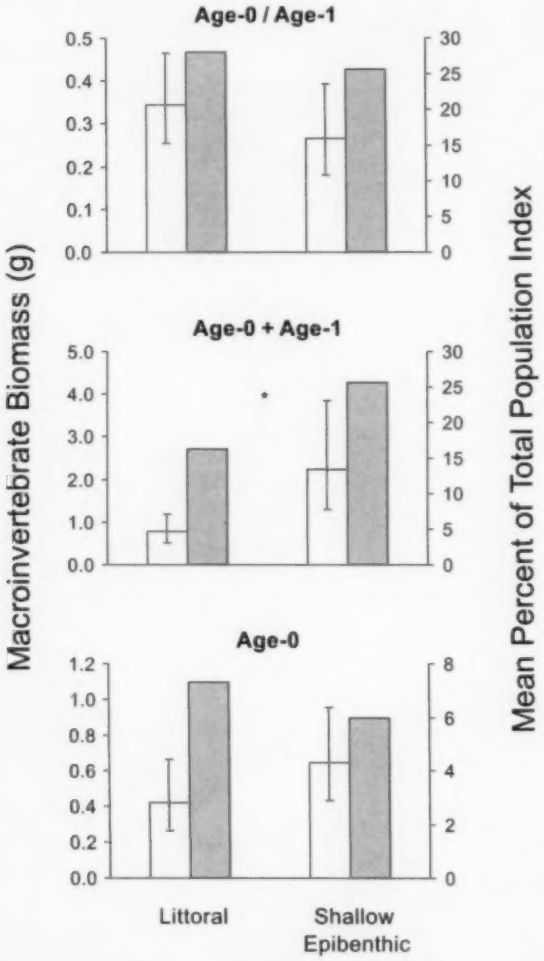
Spatial segregation among age- and size-classes of lake-dwelling fishes has been observed in many species (Werner et al. 1983*b*; Tonn et al. 1992; Langeland and L'Abée-Lund 1998). The typical pattern is that the younger age-class or age-classes inhabit the more structurally complex habitat, usually the littoral zone, and the larger size-classes use deeper benthic or pelagic habitats. The littoral habitats in lakes generally offer less risk of predation (Tabor and Wurtsbaugh 1991; Post et al. 1998, 1999) but are also often less profitable for foraging (Werner et al. 1983*a*; Naud and Magnan 1988; Tabor and Wurtsbaugh 1991). A single study observed the opposite spatial pattern of age-classes but also demonstrated that the habitat profitability was the inverse of the more common pattern (Klemetsen et al. 1989). Most authors infer that this size- or age-dependent spatial segregation is driven by the perceived risk of cannibalism or interference competition favouring the larger-bodied size-classes (Werner et al. 1983*a*, 1983*b*; Gilliam and Fraser 1987). Our

Fig. 7. Back-transformed means of total zooplankton biomass from 7 sampling days with 95% confidence intervals for each habitat per lake. Different letters indicate a significant ($p < 0.05$) difference for a pairwise comparison on log biomass using Tukey's test. LIT, littoral; SE, shallow epibenthic; DE, deep epibenthic; LIM, limnetic.



© 1999 NRC Canada

Fig. 8. Back-transformed means and 95% confidence intervals of total macroinvertebrate biomass per tile from 4 sampling days for two habitats and for each lake. An asterisk indicates a significant ($p < 0.05$) difference between habitats. Shaded bars are plotted against the right-hand axis and represent the mean percentage of the total population index of age-0 rainbow trout at dusk.



experimental manipulations involving the presence, absence, and midexperiment addition of larger-bodied conspecifics yielded spatial patterns consistent with the hypothesis that age-0 rainbow trout are plastic in their use of space in response to the spatial pattern of larger-bodied conspecifics and of zooplankton prey.

In our experiments, in the continuous presence of older and larger conspecifics, which is the natural situation, age-0 rainbow trout spatial distribution was skewed toward the littoral and benthic habitats, which likely provided a partial refuge from interactions with conspecifics. This spatial pattern has been described for natural populations of rainbow trout in lakes (Tabor and Wurtsbaugh 1991; Post et al. 1999). In addition, Spearman's rank correlation coefficients suggested that the habitat choice was not based on zooplankton abundance. The opposite spatial pattern was observed for age-0 rainbow trout in our experimental lake devoid of larger conspecifics where the largest proportion of the age-0 size-class was found in the open-water limnetic habitat, a pattern not observed in natural populations. When older and larger conspecifics are not present, the fish choose habitats based on zooplankton prey availability. In the continuous presence and continuous absence of older and larger conspecifics, there was no evidence that fish spatial distribution was correlated with macroinvertebrate abundance. Yet, in the experimental addition lake treatment, fish abundance and macroinvertebrate abundance were positively correlated. The hypotheses that spatial distribution is driven by patterns of temperature or oxygen were also not supported by the data. The differences between lakes in temperature within habitats were never greater than 2°C, suggesting that the differences in habitat choice among lakes were not based on differences in water temperature. The possibility of the distribution being driven by oxygen concentrations was rejected because concentrations were always greater than 70% of saturation in all habitats occupied by fish (Landry 1997). Therefore, our experimental results are consistent with the conceptual model of Gilliam and Fraser (1987) that small fish face a trade-off between risk of cannibalism or interference competition and habitat-specific prey profitability but that the trade-off changes through ontogeny as the young fish grow (Miller et al. 1988).

The ontogenetic component of the risk versus habitat profitability trade-off complicates interpretation of the observed temporal pattern of habitat use in the experimental lake that received larger-bodied conspecifics midexperiment. The logistic regression analysis did not show a significant effect of addition of larger-bodied conspecifics, but the temporal pattern of probability of capture success estimates is the reverse of the result in the continuous presence of conspecifics. This observation is consistent with an effect of larger-bodied conspecifics in the addition treatment but is confounded by the ontogeny of habitat use as the age-0 rainbow trout grow through a window of vulnerability with decreasing susceptibility to interference or cannibalism risks. A useful experiment to examine the ontogeny of vulnerability would be to introduce older conspecifics into a series of lakes at various times throughout ontogeny of age-0 rainbow trout. We would predict the occurrence of strong addition effects if the addition was made early in the experiment and no effects if made late in the experiment and equivocal results if the addition was done midseason, as we observed in this experiment.

The existence of an ontogenetic shift from littoral and shallow epibenthic habitats to deep epibenthic and limnetic habitats observed at dusk in the continuous presence of older conspecifics and the results from the logistic regressions suggest that the relative benefits and costs associated with the available habitats change with the size of the age-0 fish or the difference in size of the age-0 and older rainbow trout. The shift appeared more gradual, suggesting constant interference or predation pressure. Similar habitat shifts, either gradual or abrupt, have also been previously observed in young salmonids such as rainbow trout, Arctic char, *Salvelinus alpinus*, and brook trout, *Salvelinus fontinalis* (Tabor and Wurtsbaugh 1991; L'Abée-Lund et al. 1993; Venne and

© 1999 NRC Canada

Magnan 1995; Langeland and L'Abée-Lund 1998). Such size-dependent shifts in habitat use are often attributed to interference or cannibalism (Schlosser 1987; Foster et al. 1988).

The results of our experimental manipulations support the inferences drawn from observational studies that young fish alter their habitat use in response to the spatial pattern of risk of predation, cannibalism, or interference competition. Since our experiments were only partially replicated, we cannot reject a priori that the spatial patterns in age-0 rainbow trout, older conspecifics, and invertebrate prey were due to some unknown differences among our experimental lakes. This is a common problem with whole system level experiments that are designed to gain understanding of population-, community-, and ecosystem-level processes (Tonn et al. 1994; Persson et al. 1996; Post et al. 1999). Designing such experiments involves trade-offs between incorporating the appropriate spatial and temporal scales for examining processes and statistically robust experimental designs that are possible at smaller, but potentially biologically unrealistic scales (Tonn et al. 1994; Persson et al. 1996). The fact that our experiments were done at realistic spatial and temporal scales, that the size-structure treatments were allocated to experimental lakes at random, and that the fish populations had the same genetic origin and the coincidence between our results and the conceptual models of Werner et al. (1983*a*, 1983*b*), Gilliam and Fraser (1987), and others lend substantial credence to our interpretation of the importance of size-structured interactions to habitat use by age-0 rainbow trout.

The behavioural mechanisms involved in size-based interactions are better understood for stream- than for lake-dwelling fishes. Strong spatial habitat segregation within and between species of stream-dwelling salmonids results from the fact that territories are vigorously defended, the success of which is strongly body size based (Grant 1990; Marschall and Crowder 1995). In streams, small-scale variation in water velocity and prey supply rate through invertebrate drift clearly defines the costs and benefits of territory defense, the ability of which is strongly dependent on size-based aggressive abilities (Li and Brocksen 1977; Huntingford 1993). Our results, in combination with those of Post et al. (1999), suggest that lake-dwelling salmonids also have a size-dependent spatial structure based on interference competition but that the structure is distributed over larger-scale habitats defined by risk and prey profitability rather that over small-scale individually defended territories.

The effects of size-dependent interactions such as predation, interference, and exploitative competition within size-structured fish populations can have important consequences at the population level. To understand the mechanisms that govern the structure of a population, one needs to examine processes at the appropriate spatial and temporal scale to incorporate natural variability. By analyzing spatial patterns at the population scale, we developed a better understanding of the size-dependent mechanisms that modify fish behaviour in rainbow trout populations in lakes. We demonstrated with a multiple-lake experiment that older conspecifics have a direct effect on the spatial distribution of younger ones likely through risk of interference competition or cannibalism. The observed spatial restriction to a less profitable habitat early in the ontogeny of age-0 rainbow trout when larger-bodied conspecifics are present would likely reduce rates of growth and extend periods of vulnerability to aggressive encounters with conspecifics and size-selective and gape-limited piscivory. An understanding of these behavioural mechanisms is crucial if we are to understand recruitment and population-level consequences (Walters and Juanes 1993).

Acknowledgments

We gratefully acknowledge the assistance of Hugues Benoît and Craig Mushens in the field and Marianne Meding in the laboratory. The employees from the Fraser Valley Trout Hatchery, Abbostford, B.C., are also thanked for their assistance in the field and for providing all the fish. This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to J.R. Post and funds from the British Columbia Ministry of Environment, Lands and Parks to E.A. Parkinson. We thank Pierre Magnan for very useful comments on an earlier version of this manuscript.

References

Beauchamp, D.A. 1990. Seasonal and diel food habits of rainbow trout stocked as juveniles in Lake Washington. Trans. Am. Fish. Soc. **119**: 475–482.

Benoît, H.P., Post, J.R., Parkinson, E.A., and Johnston, N.T. 1998. Colonization by lentic macroinvertebrates: evaluating colonization processes using artificial substrates and appraising applicability of the technique. Can. J. Fish. Aquat. Sci. **55**: 2425–2435.

Collett, D. 1991. Modelling binary data. Chapman and Hall, London, U.K.

Eklöv, P., and Persson, L. 1996. The response of prey to the risk of predation: proximate cues for refuging juvenile fish. Anim. Behav. **51**: 105–115.

Foster, S.A., Garcia, V.B., and Town, M.Y. 1988. Cannibalism as the cause of an ontogenetic shift in habitat use by fry of the threespine stickleback. Oecologia, **74**: 577–585.

Gilliam, J.F., and Fraser, D.F. 1987. Habitat selection under predation hazard: test of a model with foraging minnows. Ecology, **68**: 1856–1862.

Grant, J.W.A. 1990. Aggressiveness and the foraging behaviour of young-of-the-year brook charr (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. **47**: 915–920.

Grant, J.W.A., and Noakes, D.L.G. 1988. Aggressiveness and the foraging mode of young-of-the-year brook charr, *Salvelinus fontinalis* (Pisces, Salmonidae). Behav. Ecol. Sociobiol. **22**: 435–445.

Hamrin, S.F., and Persson, L. 1986. Asymmetrical competition between age-classes as a factor causing population oscillations in an obligate planktivorous fish species. Oikos, **47**: 223–232.

Huntingford, F.A. 1993. Can cost-benefit analysis explain fish distribution patterns? J. Fish Biol. **43**(Suppl. A): 289–308.

Klemetsen, A., Amundsen, P.-A., Muladal, H., Rubach, S., and Solbakken, J.I. 1989. Habitat shifts in a dense, resident Arctic charr *Salvelinus alpinus* population. Physiol. Ecol. Jpn. **1**(Spec. Vol.): 187–200.

L'Abée-Lund, J.H., Langeland, A., Jonsson, B., and Ugedal, O. 1993. Spatial segregation by age and size in Arctic charr: a trade-off between feeding possibility and risk of predation. J. Anim. Ecol. **62**: 160–168.

Landry, F. 1997. Direct and indirect effects of interference competition in size-structured rainbow trout (*Oncorhynchus mykiss*) populations. Masters thesis, University of Calgary, Calgary, Alta.

© 1999 NRC Canada

Langeland, A., and L'Abée-Lund, J.H. 1998. An experimental test of the genetic component of the ontogenetic habitat shift in Arctic charr (*Salvelinus alpinus*). Ecol. Freshwater Fish, **7**: 200–207.

Li, H.W., and Brocksen, R.W. 1977. Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). J. Fish Biol. **11**: 329–341.

Magnan, P., and FitzGerald, G.J. 1984. Ontogenetic changes in diel activity, food habits and spatial distribution of juvenile and adult creek chub, *Semotilus atromaculatus*. Environ. Biol. Fishes, **11**: 301–307.

Marschall, E.A., and Crowder, L.B. 1995. Density-dependent survival as a function of size in juvenile salmonids in streams. Can. J. Fish. Aquat. Sci. **52**: 136–140.

McCarthy, I.D., Carter, C.G., and Houlihan, D.F. 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. **41**: 257–263.

Miller, T.J., Crowder, L.B., Rice, J.A., and Marschall, E.A. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657–1670.

Naud, M., and Magnan, P. 1988. Diel onshore–offshore migrations in northern redbelly dace, *Phoxinus eos* (Cope), in relation to prey distribution in a small oligotrophic lake. Can. J. Zool. **66**: 1249–1253.

Northcote, T.G. 1969. Patterns and mechanisms in lakeward migratory behaviour of juvenile rainbow trout. *In* Symposium on salmon and rainbow trout in streams. *Edited by* T.G. Northcote. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C. pp. 183–203.

Olson, R.A., Winter, J.D., Nettles, D.C., and Hayes, J.M. 1988. Resource partitioning in summer by salmonids in south-central Lake Ontario. Trans. Am. Fish. Soc. **117**: 552–559.

Persson, L., and Eklöv, P. 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology, **76**: 70–81.

Persson, L., Andersson, J., Wahlström, E., and Eklöv, P. 1996. Size-specific interactions in lake systems: predator gape limitation and prey growth rate and mortality. Ecology, **77**: 900–911.

Polis, G.A. 1981. The evolution and dynamics of intraspecific predation. Annu. Rev. Ecol. Syst. **12**: 225–251.

Post, J.R, Parkinson, E.A., and Johnston, N.T. 1998. Spatial and temporal variation in risk to piscivory of age-0 rainbow trout: patterns and population consequences. Trans. Am. Fish. Soc. **127**: 932–942.

Post, J.R, Parkinson, E.A., and Johnston, N.T. 1999. Density-dependent processes in structured fish populations: assessment of interaction strengths in whole lake populations. Ecol. Monogr. **69**: 155–175.

Rosenau, M.L. 1991. Natal-stream rearing in three populations of rainbow trout in Lake Taupo, New Zealand. N.Z. J. Mar. Freshwater Res. **25**: 81–91.

Schlosser, I.J. 1987. The role of predation in age- and size-related habitat use by stream fishes. Ecology, **68**: 651–659.

Schoener, T.W. 1983. Field experiments on interspecific competition. Am. Nat. **122**: 240–285.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Company, New York.

Steinberg, D., and Colla, P. 1991. LOGIT: a supplementary module for SYSTAT. SYSTAT Inc., Evanston, Ill.

Tabor, R.A., and Wurtsbaugh, W.A. 1991. Predation risk and the importance of cover for juvenile rainbow trout in lentic systems. Trans. Am. Fish. Soc. **120**: 728–738.

Tonn, W.M., Paszkowski, C.A., and Holopainen, I.J. 1992. Piscivory and recruitment: mechanisms structuring prey populations in small lakes. Ecology, **73**: 951–958.

Tonn, W.M., Holopainen, I.J., and Paszkowski, C.A. 1994. Density-dependent effects and the regulation of crucian carp populations in single-species ponds. Ecology, **75**: 824–834.

Venne, H., and Magnan, P. 1995. The impact of intra- and interspecific interactions on young-of-the-year brook charr, in temperate lakes. J. Fish Biol. **46**: 669–686.

Walters, C.J., and Juanes, F. 1993. Recruitment limitation as a consequence of natural selection for use of restricted feeding habitats and predation risk taking by juvenile fishes. Can. J. Fish. Aquat. Sci. **50**: 2058–2070.

Warner, E.J., and Quinn, T.P. 1995. Horizontal and vertical movements of telemetered rainbow trout (*Oncorhynchus mykiss*) in Lake Washington. Can. J. Zool. **73**: 146–153.

Werner, E.E., and Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. Annu. Rev. Ecol. Syst. **15**: 393–425.

Werner, E.E., Mittelbach, G.G., Hall, D.J., and Gilliam, J.F. 1983*a*. Experimental tests of optimal habitat use in fish: the role of relative habitat profitability. Ecology, **64**: 1525–1539.

Werner, E.E., Gilliam, J.F., Hall, D.J., and Mittelbach, G.G. 1983*b*. An experimental test of predation risk on habitat use in fish. Ecology, **64**: 1540–1548.

© 1999 NRC Canada

Effects of iron limitation and aquatic humic substances on the growth of *Microcystis aeruginosa*

Akio Imai, Takehiko Fukushima, and Kazuo Matsushige

Abstract: Aquatic humic substances (AHSs) from the eutrophic Lake Kasumigaura, Japan, significantly inhibited the growth of *Microcystis aeruginosa* at a concentration level similar to that found in the lake. AHSs were isolated and quantified using XAD-8 resin. The effects of these isolated AHSs on the growth of *M. aeruginosa* cultures were evaluated in a chemically defined medium. The production of siderophores, iron-specific complexing agents, of *M. aeruginosa* under iron limitation was also examined. Fulvic acid (the lower molecular size fraction of the AHSs) accounted for more than 85% of AHSs in Lake Kasumigaura. Under iron limitation, *M. aeruginosa* produced hydroxamate-type siderophores; however, they were not effective in enabling substantial recovery of growth. *Microcystis aeruginosa* growth was significantly inhibited when fulvic acid was 2 mg dry weight·L^{-1}, similar to the concentration in the lake, even when siderophores were produced. Growth inhibition was probably due to a deficiency of iron caused by iron complexation with the fulvic acid. It was suggested that AHSs play an important role in formation and disappearance of *M. aeruginosa* blooms in eutrophic lakes.

Résumé : Les substances humiques aquatiques (SHA) du lac eutrophe Kasumigaura inhibent de façon appréciable la croissance de *Microcystis aeruginosa* à des concentrations semblables à celles notées dans le lac. Les SHA ont été isolées et quantifiées sur résine XAD-8. Les effets de ces isolats sur la croissance de *M. aeruginosa* ont été évalués par culture sur des milieux chimiquement définis. La production de sidérophores, des agents complexants spécifiques au fer, de *M. aeruginosa* en condition de limitation par le fer a aussi été examinée. L'acide fulvique (la fraction de masse moléculaire des SHA) représentait plus de 85 % des SHA du lac Kasumigaura. En condition de limitation par le fer, *M. aeruginosa* produisait des sidérophores de type hydroxamate, mais ces derniers ne permettaient pas d'obtenir un rétablissement appréciable de la croissance. La croissance de *M. aeruginosa* était fortement inhibée à une concentration d'acide fulvique de 2 mg (poids sec) L^{-1}, une valeur semblable à celle notée dans le lac, même si des sidérophores étaient produits. L'inhibition de la croissance s'expliquait sans doute par une carence de fer découlant de la formation de complexes de fer avec l'acide fulvique. Il est proposé que les SHA jouent un rôle important dans l'apparition et la disparition des pullulations de *M. aeruginosa* dans les lacs eutrophes.

[Traduit par la Rédaction]

Introduction

In oxic natural waters, aquatic organisms are constantly faced with the problem of how to overcome the extremely low solubility of Fe(III) (log K_{so} = –38.7, Stumm and Morgan 1981) to meet their cellular demands for iron. To alleviate the problem of low iron availability, cyanobacteria synthesize specific iron-complexing agents, low molecular size compounds called siderophores, that enable them to solubilize, and therefore acquire, the Fe(III) present as (oxy)hydroxides (Neilands 1981). Cyanobacteria have higher cellular iron requirements than other algae (Brand 1991), and increased iron concentrations are reported to selectively stimulate cyanobacterial growth over competing chlorophytes. Siderophore-mediated iron uptake is believed to be a contributing factor in their ability to dominate eukaryotic algae (Murphy et al. 1976). The main constituents of cyanobacterial siderophores are hydroxamates (Boyer et al. 1987).

In aquatic environments, in addition to Fe(III)-specific complexing ligands such as siderophores, there exist nonspecific complexing ligands that are involved in Fe(III) speciation. Aquatic humic substances (AHSs) are typical nonspecific naturally occurring ligands, constituting 30–80% of dissolved organic carbon (DOC). They are the largest fraction of natural organic matter in most waters and are composed of fulvic acid and humic acid (Thurman 1985). Fulvic acid is the low molecular size fraction of AHSs and humic acid is the high molecular size fraction. They are straw-colored, polar, organic acids that are derived from soil humus, terrestrial and aquatic plants, and plankton. AHSs are known to form soluble complexes with metal ions (Kerndorff and Schnizer 1980) and have been reported to have a variable effect on the availability of iron to phytoplankton. The occurrence of phytoplankton blooms has

Received October 22, 1998. Accepted July 6, 1999.
J14854

A. Imai[1] **and K. Matsushige.** National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305, Japan.
T. Fukushima. Department of Civil Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739, Japan.

[1]Author to whom all correspondence should be addressed.
e-mail: aimai@nies.go.jp

© 1999 NRC Canada

been reported to correlate with the levels of AHSs in the water (Ingle and Martin 1971). Stimulation of $H^{14}CO_3^-$ uptake by high levels of humic acids has also been reported (Prakash et al. 1973). On the other hand, several authors have found that AHSs appeared to restrict primary production by making iron or some other metal unavailable (Jackson and Hecky 1980; Guildford et al. 1987). AHSs seem to have a significant effect on the growth of phytoplankton, whether it be positive or negative.

Thus, it is reasonable to suggest that iron availability may significantly affect cyanobacteria dominance in lake water and that both siderophores and AHSs play an important role by complexation in controlling iron supply to cyanobacteria. To date, there are no studies that have examined both siderophore production by cyanobacteria and the effect of AHSs on cyanobacterial growth under iron limitation. Furthermore, there have been no studies that have isolated AHSs from lake water in order to evaluate the effects of AHSs on the growth of bloom-forming cyanobacteria that inhabit that particular lake. The use of AHSs isolated from actual lake water is of importance because there are significant differences in physiochemical characteristics among commercial humic substances and natural humic substances of different origin (Malcolm 1990).

It has been well documented that free metal ion activity rather than the total metal concentration controls biological availability and toxicity of metals to phytoplankton (Anderson and Morel 1982). However, recent evidence suggests that free hydrated ferric iron (Fe^{3+}) is not likely to be a biologically important species due to its exceedingly low concentration and slow water exchange rate. Dissolved hydrolyzed ferric species such as $FeOH^{2+}$, $Fe(OH)_2^+$, $Fe(OH)_3^0$, and $Fe(OH)_4^-$ may be biologically active species (Hudson et al. 1992). Thus, the sum of free hydrated and hydrolyzed ferric iron species (FHFe) rather than Fe^{3+} is appropriate to represent the biologically available Fe(III) concentration in our study.

Our aim in the present study was to examine effects of AHSs on the growth of cyanobacteria under iron-limiting conditions. The specific objectives were to quantify and isolate AHSs present in eutrophic Lake Kasumigaura, Japan, examine siderophore production in cultures of the freshwater cyanobacterium *Microcystis aeruginosa* (isolated from algal blooms in Lake Kasumigaura) grown in a chemically defined medium where the FHFe concentration was controlled, and determine if AHSs stimulate or inhibit the growth of *M. aeruginosa*.

Materials and methods

Collection and pretreatment of lake water samples

Lake Kasumigaura, the second largest lake in Japan, is located in the eastern part of the Kanto Plain, 60 km northeast of Tokyo. It is a shallow eutrophic lake (surface area 167.7 km, maximum depth 7.3 m, mean depth 3.9 m) where *Microcystis* bloomed every summer until 1986 and then suddenly disappeared after 1987 (Takamura et al. 1992). Since then, filamentous cyanobacteria such as *Oscillatoria* have become dominant instead of *Microcystis*. Essential trace elements such as iron have been suspected of being a limiting nutrient for *M. aeruginosa*. The average dissolved iron concentration in the surface water at the center of Lake Kasumigaura was reported to be about 10^{-7} M (Inaba et al. 1997).

To isolate and quantify AHSs, surface water of Lake Kasumigaura was collected at a sampling station of the Lake Research Laboratory of the National Institute for Environmental Studies (NIES) from March 1993 to February 1994. The station was located 150 m offshore. For AHSs isolation, about 180 L of lake water was collected in 20-L high-density polyethylene containers prewashed with 6 M HCl. The water was then filtered through three glassfiber filter cartridges connected in series (Bolston LP-20, LP-50, and LP-80) with a diaphragm pump whose water-contacting parts were all made of Teflon. The final cartridge captured 0.22-μm particles at 80% efficiency. For quantitative analysis, samples were collected in precombusted glass bottles (450°C for 4 h) and filtered through a 0.45-μm membrane filter.

Isolation and quantification of AHSs

AHSs were isolated from Lake Kasumigaura water samples by adsorption onto Amberlite XAD-8 resin (20–60 μm mesh size) according to the procedure developed by Thurman and Malcolm (1981). About 150 L of the filtrate was acidified to pH 2.0 with 6 M HCl and then passed through a glass column containing 1.2 L of XAD-8 resin with a peristaltic pump at a flow rate of 15 bed volumes·h^{-1}. The column was eluted with 0.1 M NaOH in the reverse direction. The elutant was again acidified to pH 2.0 and recycled through XAD-8 resin (70 mL wet volume). After elution with 0.1 M NaOH, the elutant was acidified to pH 1.0, allowed to settle for 24 h to precipitate humic acid, and centrifuged (about 10 000 × *g*) to separate humic acid precipitate from fulvic acid in solution. The DOC concentration was measured before and after centrifugation to determine the ratio of fulvic acid to AHSs (= fulvic acid + humic acid). For growth experiments, fulvic acid was isolated on May 28, 1993, by readsorbing onto a third column of 20 mL of XAD-8 resin, rinsing with Milli-Q water (Milli-Q SP. TOC, Millipore), and eluting with 0.1 M NaOH. The fulvic acid was hydrogen saturated by passing through a macroporous cation-exchange resin (Bio-Rad Lab., AG-MP-50 hydrogen form) and freeze-dried.

The concentration of AHSs in Lake Kasumigaura was quantified by a simple DOC fractionation method using XAD-8 resin (Leenher 1981). Resin cleanup and conditioning were the same as those used for the isolation procedure (Thurman and Malcolm 1981). The filtrate of the lake water was acidified to pH 2.0 with 6 M HCl. Then, 200 mL of the filtrate was passed through a glass column containing 3 mL wet volume of XAD-8 resin by a peristaltic pump at a flow rate not exceeding 1.5 mL·min^{-1}. The column capacity factor, k', was 50. Subsequently, one bed volume of 0.01 M HCl was passed through the column. The column was then eluted in the reverse direction with three or four bed volumes of 0.1 M NaOH at a flow rate of less than 1 mL·min^{-1}. The elutant volume and DOC were measured. The concentration of AHSs was calculated by multiplying the elutant DOC by the elutant to sample volume ratio. The bleeding from XAD-8 resin in 0.1 M NaOH elutant was less than 1.0 mg DOC·L^{-1}. The concentration of AHSs may be overestimated by no more than 0.06 mg DOC·L^{-1}.

All columns were constructed of glass with Teflon end-caps and fittings. Connecting tubing was Teflon and peristaltic pump tubing was Tygon. DOC measurements were made as nonpurgeable organic carbon with a Shimadzu TOC-5000 total organic carbon analyzer. At least triplicate measurements were made for each sample and analytical precision was typically ±1%. Milli-Q water was used throughout the preparation of HCl and NaOH solutions.

Cyanobacterial strain and medium conditions

Axenic unialgal cultures of *M. aeruginosa* (NIES-98) were selected because this cyanobacterial strain was isolated from an algal bloom in Lake Kasumigaura and has been extensively studied. The stock culture was obtained from the NIES Microbial Culture Collection. *Microcystis aeruginosa* was maintained in borosilicate

© 1999 NRC Canada

Table 1. Composition of modified CB medium.

Substrate	Concentration (M)
$Ca(NO_3)\cdot 4H_2O$	6.35×10^{-4}
KNO_3	9.89×10^{-4}
K_2HPO_4	5.74×10^{-4}
$MgSO_4\cdot 7H_2O$	1.62×10^{-4}
$Na_2EDTA\cdot 2H_2O$	1.00×10^{-5}
$MnCl_2\cdot 2H_2O$	5.46×10^{-7}
$ZnSO_4\cdot 7H_2O$	2.30×10^{-7}
$CoCl_2\cdot 6H_2O$	5.04×10^{-8}
$Na_2MoO_4\cdot 4H_2O$	3.09×10^{-8}
Bicine ($(HOCH_2CH_2)_2 = NHCH_2COOH$)	3.06×10^{-3}
$FeCl_3\cdot 6H_2O$	$10^{-5.56}$, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}
Vitamin B_{12}	7.4×10^{-11} (0.1 $\mu g\cdot L^{-1}$)
Biotin	4.1×10^{-10} (0.1 $\mu g\cdot L^{-1}$)
Thiamine HCl	3.0×10^{-8} (10 $\mu g\cdot L^{-1}$)

Note: Milli-Q water was used throughout the preparation of the medium solution. The pH of the medium was adjusted to 9 with HCl and NaOH.

glass culture tubes at 25°C and about 100 μeinsteins$\cdot m^{-2}\cdot s^{-1}$ under a 12 h light : 12 h dark cycle on CB medium (Watanabe and Nozaki 1994).

For the growth experiments, the FHFe concentration was controlled with trace metal ion buffers of ferric iron salts and EDTA. The medium used in the growth experiments was a modified CB medium (Table 1). Departures from the standard CB medium included the change in concentration of EDTA to 1.00×10^{-5} M instead of 0.80×10^{-5} M and the replacement of β-glycerophosphate with K_2HPO_4 (5.74×10^{-4} M). The latter change was necessary, since stability constants for complexation reactions between ferric iron species and β-glycerophosphate are unavailable. Concentrations of $FeCl_3\cdot 6H_2O$ were altered from $10^{-5.6}$ to 10^{-11} M to vary the FHFe concentration ($10^{-7.4}$ to $10^{-13.9}$ M). The major ion solutions and macronutrient solutions were treated with Chelex-100 (Bio-Rad Lab.) to remove residual iron (Morel et al. 1979). The Chelex-100 resin was precleaned through Soxhlet extraction with methanol for 24 h and extensively rinsed with Milli-Q water. The total dissolved iron concentration in the lower-iron media was checked by a graphite furnace atomic absorption spectrometer (Perkin-Elmer Z-5100A, detection limit 1.8×10^{-9} M).

Cells for the inoculum were precultured in batch cultures at or near the experimental iron level. These cultures were transferred twice to ensure that they were close to experimental iron levels. All glass culture tubes, flasks, and glassware were soaked in 3 M HCl for 48 h and rinsed thoroughly with Milli-Q water.

The MINEQL computer program was used to predict the FHFe concentration in the medium (Westall et al. 1976), with stability constants of the metal–bicine complexes from Smith and Martel (1975).

Growth experiments

Growth experiments of *M. aeruginosa* were conducted in batch cultures. Cells were inoculated into 200 mL of the modified CB medium in 500-mL Erlenmeyer flasks and grown axenically under continuous light (about 100 μeinsteins$\cdot m^{-2}\cdot s^{-1}$) at 25 ± 0.2°C. The flasks were shaken by hand once a day. Chlorophyll *a* (Chl *a*) concentrations were determined with time. Hydroxamate-type siderophore activity in the filtrate of the culture medium was measured after Chl *a* concentrations had reached a stationary phase. Growth experiments were run in duplicate or triplicate for each FHFe level.

In a like manner, growth experiments for the evaluation of the effects of AHSs on the growth of *M. aeruginosa* were conducted using the fulvic acid isolated from Lake Kasumigaura on May 28, 1993. The experiment at the lowest FHFe of $10^{-13.9}$ M was not conducted, since the growth was unstable and no stationary phase was observed in the growth experiment without the fulvic acid. Two concentration levels of fulvic acid were selected: 2 and 10 mg dry weight (DW)$\cdot L^{-1}$. Fulvic acid at 2 mg DW$\cdot L^{-1}$ corresponds to about the average AHS concentration in the lake during 1993–1994 (see Results section). The stock solution of the fulvic acid was prepared by dissolving the freeze-dried fulvic acid into Milli-Q water with a minimal amount of NaOH and adjusting the pH to around 9. The stock solution was filter-sterilized with a 0.45-μm membrane filter. The medium was spiked with the required amounts of the stock solution and allowed to equilibrate overnight before inoculation. Chl *a* concentrations were measured along with time, and hydroxamate-type siderophore concentrations were determined in the filtrate of the culture medium at the end of the growth experiments. Because of the scarcity of the isolated fulvic acid, each experiment was done once.

Chl *a* concentrations were determined by a fluorometric method. Chl *a* of the samples collected on Whatman GF/C glassfiber filters was extracted with 100% methanol in a refrigerator overnight. Fluorescence intensity of Chl *a* was measured with a Shimadzu RF-5000 spectrofluorometer using certified Chl *a* (Sigma Chemical Co.) for calibration. The excitation wavelength was 430 nm; the emission was measured at 663 nm. The detection limit was about 0.04 μg Chl $a\cdot L^{-1}$.

Hydroxamate-type siderophore activity in the *M. aeruginosa* culture medium was measured using the modified Csaky method (Gillam et al. 1981). After the cells had reached the stationary phase, the medium was centrifuged (3000 × *g* for 20 min) and filtered through a 0.45-μm membrane filter. Twenty millilitres of the cell-free filtrate was freeze-dried and brought up to 4 mL with Milli-Q water prior to the colorimetric analysis. Hydroxylamine hydrochloride was used as a standard. Absorbance of the diazo-coupled product was measured at 543 nm with a Shimadzu UV-2200 spectrophotometer using a 100-mm quartz cuvette. The detection limit was about 17 μg $NH_2OH\text{-}N\cdot L^{-1}$.

Results

AHSs in Lake Kasumigaura

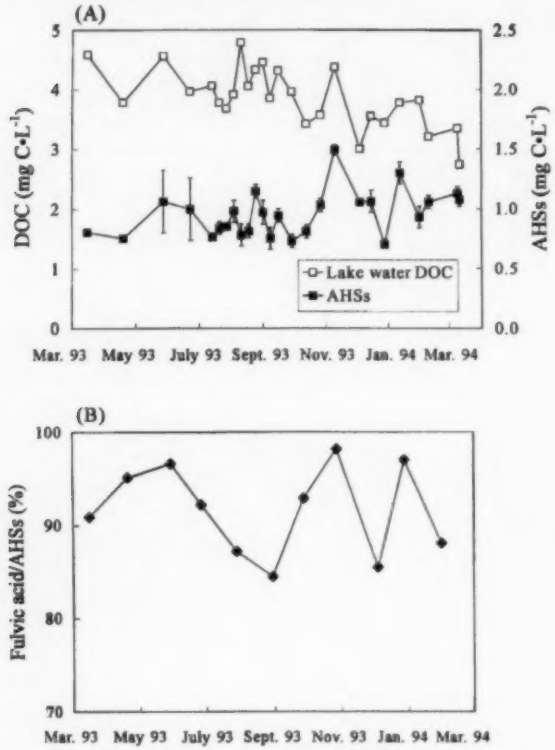
AHSs in Lake Kasumigaura ranged from 0.75 to 1.49 mg $C\cdot L^{-1}$ and averaged 0.96 mg $C\cdot L^{-1}$ during this study from March 1993 to February 1994 (Fig. 1A). The AHS concentration did not vary much, although reported to do so in other lakes (Tipping and Woof 1983), but remained relatively constant from spring to fall and then increased to a maximum in November. The lake water DOC tended to decline over the study period, ranging from 2.74 to 4.79 mg $C\cdot L^{-1}$ with an average concentration of 3.86 mg $C\cdot L^{-1}$.

At this sampling station, Chl *a* and particulate organic carbon (POC) have been routinely monitored (Fukushima 1995). Their correlations with AHSs were examined. AHSs were not significantly correlated with DOC, POC, or Chl *a* (*t* test, $P > 0.05$). The only significant correlation with AHSs was observed for Chl *a* concentration during the summer (July–September inclusive) ($r = 0.75$, $P < 0.005$), implying that the production of AHSs may be related to phytoplankton activity during summer.

AHSs accounted for 16.3–39.3% of the DOC in Lake Kasumigaura (Fig. 1A). The percent fraction tended to increase from spring to winter, showing a large variation. More than 85% of AHSs in Lake Kasumigaura consisted of

© 1999 NRC Canada

Fig. 1. (A) Variations in DOC and AHSs in Lake Kasumigaura from March 1993 to February 1994. Error bars represent ±1 SD of the mean. (B) Variations in the percent fraction of fulvic acid in AHSs (= fulvic acid + humic acid) in Lake Kasumigaura from March 1993 to February 1994. DOC measurements were made in duplicate for each sample using its subfraction. Relative deviation from the mean ranged from 0.9 to 2.7%.


(A)
DOC (mg C·L⁻¹)
AHSs (mg C·L⁻¹)
Lake water DOC
AHSs
Mar. 93 May 93 July 93 Sept. 93 Nov. 93 Jan. 94 Mar. 94
(B)
Fulvic acid/AHSs (%)
Mar. 93 May 93 July 93 Sept. 93 Nov. 93 Jan. 94 Mar. 94

fulvic acid; low molecular size fulvic acid dominated over high molecular size humic acid (Fig. 1B).

Growth and siderophore production of *M. aeruginosa*

Iron in the 10^{-10} M Fe ($10^{-12.9}$ M FHFe) and 10^{-11} M Fe ($10^{-13.9}$ M FHFe) media as well as in Milli-Q water was not detected (detection limit 1.8×10^{-9} M). van den Berg (1995) reported that the iron blank due to the reagents prepared with Milli-Q water was less than 2×10^{-11} M. Thus, Milli-Q water may contain iron at less than about 10^{-11} M. The Chelex-treated solutions used also have iron near the detection limit. This is comparable with that reported in the literature, as described below. Hudson et al. (1992) stated that synthetic seawater medium, prepared with Milli-Q using Chelex-100, contained less than 10^{-9} M Fe. In preparing the medium, the Chelex-treated solutions were diluted by 50 times; their contribution to the medium may be about 2×10^{-11} M Fe. Down to 10^{-10} M Fe, the iron level in the medium may have been achieved as designed. However, there is still a great possiblity that the iron level at 10^{-11} M Fe was similar to that at 10^{-10} M Fe.

In all but one of the growth experiments, the Chl *a* concentration of *M. aeruginosa* increased exponentially with time, showing no lag time, and reached a stationary phase (Fig. 2). The single exception was the set at pFHFe (= –log[FHFe]) 13.9 where the Chl *a* concentration increased exponentially for only 6 days up to 1.2 μg·L^{-1} and then decreased abruptly, exhibiting no stationary phase.

The initial slope of the plot of log Chl *a* versus time was found, as expected, to vary significantly with increasing pFHFe (decreasing FHFe); however, traditional nutrient-limited growth kinetics were not exhibited. The initial slope did not decrease in a simple linear fashion with increasing pFHFe. Neither did the concentration of Chl *a* in the stationary phase (Fig. 3). From pFHFe 7.4 to 10.9, the stationary-phase Chl *a* declined exponentially from 2914 to 106 μg·L^{-1} and then, at pFHFe 11.9, recovered substantially to 366 μg·L^{-1}. Even at pFHFe 12.9, the Chl *a* concentration was about 13% greater than that at pFHFe 10.9.

Specific growth rates (μ) were estimated by dividing the slope in the exponential growth phase by ln 2 (0.693) (Fig. 4). The μ at pFHFe 7.4 was 0.56 ± 0.06·day^{-1}. This value was comparable with that observed in the standard CB medium (0.54 ± 0.01·day^{-1}) as well as with that reported under iron-replete conditions (0.55·day^{-1}). This clearly shows that at this pFHFe, the iron supply to *M. aeruginosa* was sufficient. The μ decreased linearly by about 70% as the pFHFe was increased from 7.4 to 10.9, significantly recovered at pFHFe 11.9, and then decreased at pFHFe 12.9.

The production of hydroxamate-type siderophores by *M. aeruginosa* under conditions of iron limitation was confirmed; the siderophore production corresponded with recovery in both μ and stationary Chl *a* concentration (Figs. 3 and 4). When available iron was sufficient at pFHFe 7.4, siderophore production per unit weight of Chl *a* was negligible at 0.03 ± 0.02 μg NH_2OH-N·μg Chl a^{-1}. As the pFHFe was increased (available iron became limiting), siderophore production increased gradually up to pFHFe 10.9, accompanying significant reductions both in μ and stationary Chl *a* concentration. At pFHFe 11.9, siderophore production started to increase further with a slight recovery of and a substantial increase in stationary Chl *a* concentration, and then, at pFHFe 12.9, siderophore production dramatically increased to 1.6 ± 0.15 μg NH_2OH-N·μg Chl a^{-1}. The concentration of siderophores in the medium at pFHFe 12.9 was about five times greater than that at pFHFe 7.4 (Table 2).

The siderophore production at pFHFe 13.9 is not given in Fig. 4, since there was no observed stationary phase in terms of the Chl *a* concentration (Fig. 2). However, the ratio of the siderophore production in the medium to the maximum Chl *a* was 54 ± 27 μg NH_2OH-N·μg Chl a^{-1}, which is far greater than that at pFHFe 12.9. Since the affinity of siderophores toward Fe(III) could be exceedingly great, the Fe(III) source may be too low to sustain the growth of *M. aeruginosa* at this pFHFe, although siderophore-mediated uptake was active. It is possible that the cells growing at this FHFe level were able to carry on exponential growth for 5 days using only intracellular stores of iron, even though this is speculative.

The interrelationship among μ, stationary Chl *a* concentration, and production of hydroxamate-type siderophores with varying FHFe clearly demonstrates that *M. aeruginosa* induces a high-affinity iron transport system under conditions

© 1999 NRC Canada

Fig. 2. Typical time course of Chl *a* of *M. aeruginosa* in batch cultures as a function of the concentration of free and hydrolyzed ferric iron species (FHFe) (pFHFe = –log[FHFe]).

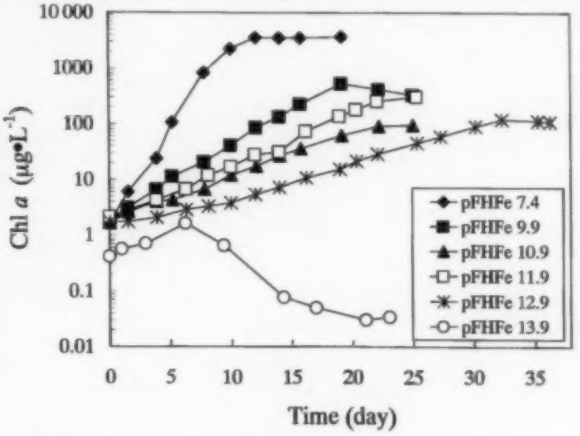
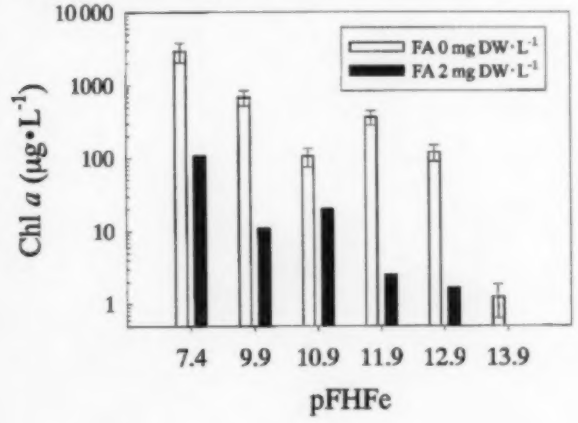


Fig. 3. Stationary phase and maximum Chl *a* concentrations of *M. aeruginosa* in batch cultures in the absence and presence of fulvic acid at 2 mg DW·L^{-1}, respectively, as a function of the concentration of free and hydrolyzed ferric iron species (FHFe). In the absence of fulvic acid, at pFHFe (= –log[FHFe]) 13.9, the maximum Chl *a* concentration reached is shown. Error bars represent ±1 SD of the mean. In the presence of fulvic acid, each experiment was done once. At pFHFe 12.9, the initial Chl *a* is shown, and at pFHFe 13.9, the data are missing.

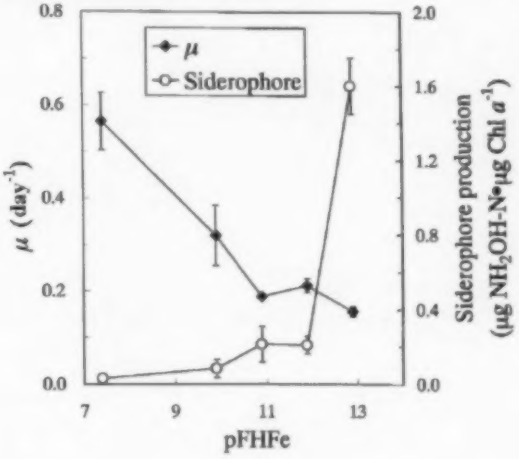


of limiting iron availability. FHFe concentrations of $10^{-11.9}$ M or lower are required to induce this iron transport system.

Effects of fulvic acid on *M. aeruginosa*

Fulvic acid isolated from Lake Kasumigaura water showed a significant negative effect on the growth of *M. aeruginosa* cultured under both iron-limited and iron-replete conditions (Fig. 5). The extent of the negative effect was dependent on the fulvic acid concentration. When fulvic acid was added to the medium at 10 mg DW·L^{-1}, *M. aeruginosa* did not grow at all, whether iron limited or iron replete. When the fulvic acid was reduced to 2 mg

Fig. 4. Relationship between siderophore production and specific growth rate (μ) of *M. aeruginosa* as a function of the concentration of free and hydrolyzed ferric iron species (FHFe). Error bars represent ±1 SD of the mean.



DW·L^{-1}, growth of *M. aeruginosa* was observed for all pFHFes except for 12.9 where the Chl *a* concentration declined without showing any growth. At pFHFes of 7.4, 9.9, and 10.9, no lag phase was observed and the initial slopes of the growth curves were similar to those without the addition of the fulvic acid. At pFHFe 11.9, however, the value of the initial slope declined significantly to about 45% of that of the slope in the absence of the fulvic acid. An abrupt reduction in Chl *a* concentration took place in the middle of the exponential phase after around 8, 5, 12, and 4 days at pFHFes of 7.4, 9.9, 10.9, and 11.9, respectively, and no stationary phase was observed. In addition, the observed maximum Chl *a* concentration showed a tendency to decrease as the pFHFe was increased (Fig. 3). However, the maximum Chl *a* concentration at pFHFe 10.9 was about double that at pFHFe 9.9. This is in stark contrast with the culture grown without the fulvic acid, in which the stationary Chl *a* concentration at pFHFe 10.9 was about nine times smaller than that at pFHFe 9.9. The duration of the exponential growth phase and the maximum Chl *a* concentration did not correspond linearly to the FHFe concentration. In the presence of fulvic acid at 2 mg DW·L^{-1}, as was the case without the fulvic acid, traditional nutrient-limited growth kinetics were not exhibited.

Siderophore production was also dependent on the fulvic acid concentration; however, the effect of the FHFe concentration was not clearly seen. With fulvic acid added at 10 mg DW·L^{-1}, siderophores were not detected in the cell-free culture medium at any pFHFe (the detection limit in this study was about 17 μg NH_2OH-N·L^{-1}) (Table 2). When the medium contained fulvic acid at 2 mg DW·L^{-1}, siderophores were detected at 20–48 μg NH_2OH-N·L^{-1} in the media with pFHFes of 7.4–11.9. At pFHFe 12.9, no siderophores were detected. The siderophore concentration at pFHFe 7.4, the greatest FHFe concentration, was considerably low, barely greater than the detection limit. No clear trend was recognized in the siderophore concentration with varying FHFe

© 1999 NRC Canada

Fig. 5. Effects of fulvic acid on the growth of batch cultures of *M. aeruginosa*: □, 0 mg DW·L^{-1}; ■, 2 mg DW·L^{-1}; ▲, 10 mg DW·L^{-1}. pFHFe (= –log[FHFe]) = (A) 7.4, (B) 9.9, (C) 10.9, (D) 11.9, and (E) 12.9.

Table 2. Hydroxamate-type siderophore concentration (μg NH_2OH-N· L^{-1}) (SD in parentheses) in culture filtrate.

pFHFe (= –log[FHFe])	Fulvic acid concentration (mg DW·L^{-1})		
	0	2	10
7.4	42 (4.0)	20	nd
9.9	48 (8.7)	48	nd
10.9	27 (6.4)	38	nd
11.9	69 (13.2)	48	nd
12.9	192 (36.6)	nd	nd
13.9	58 (21.4)	—	—

Note: nd, not detected.

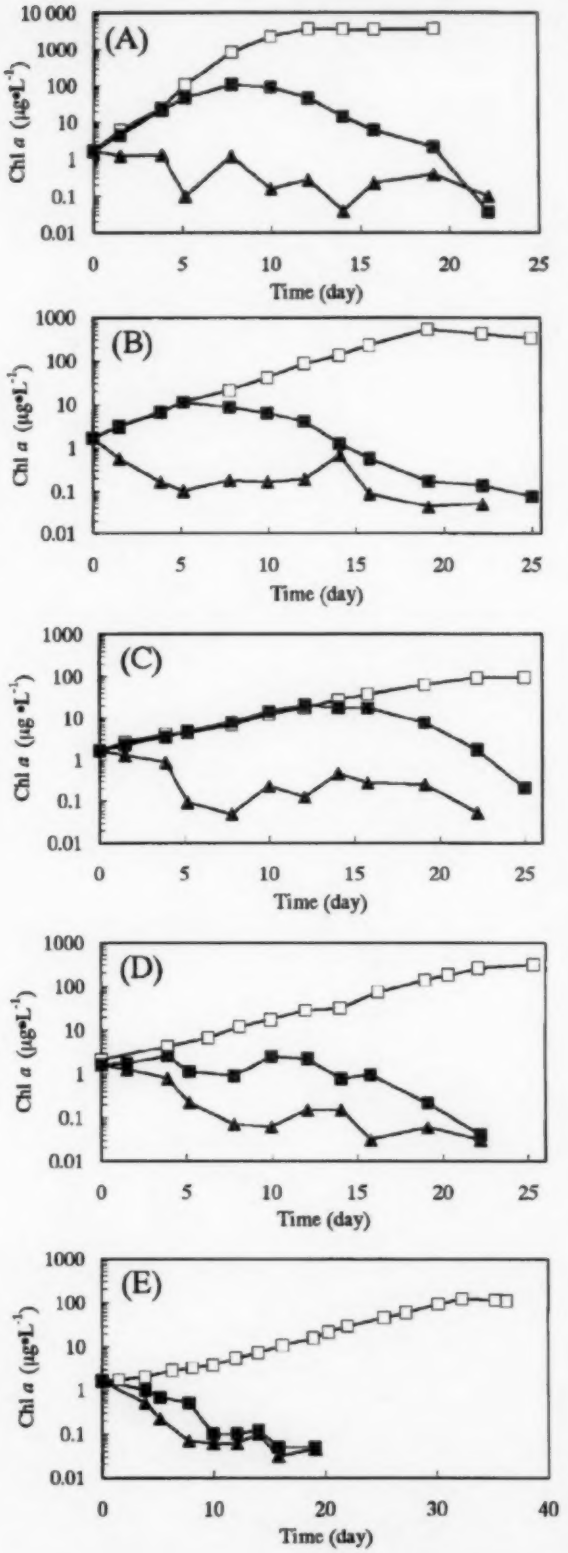
concentrations. Compared with the sets without the fulvic acid, the siderophore concentration decreased substantially except for the set at pFHFe 10.9, where the siderophore concentration was slightly increased. This may result in elongation of the exponential growth phase and the greater maximum Chl *a* concentration at this pFHFe.

To ascertain whether the fulvic acid caused analytical interference in the determination of siderophores, additional experiments were done using deferoxamine mesylate (DFO) (Sigma Chemical Co.), a typical hydroxamate-type siderophore. Siderophore concentration was measured for Milli-Q water, the DFO solution (about 1340 μg NH_2OH-N·L^{-1}), the fulvic acid solution (10 mg DW·L^{-1}), and the mixture of DFO and fulvic acid (equal in concentration to their respective solutions). Siderophore meaurements were made in quadruplicate for each case. We found that there was no significant difference between the DFO solution and the mixture of DFO and fulvic acid (ANOVA, $P > 0.3$) and Milli-Q water and the fulvic acid soltuion (ANOVA, $P > 0.8$). Thus, it is likely that fulvic acid does not interfere with the colorimetirc determination of hydroxamate-type siderophores by the Csaky method.

Discussion

The contribution of AHSs to the DOC in Lake Kasumigaura was 16–40%. This contrasts with results from typical temperate lakes where AHSs account for 30–60% of the DOC (Thurman 1985). In lakes where algal material is the main source of DOC, AHSs were reported to account for 13–20% (McKnight et al. 1994). Thus, in eutrophic Lake Kasumigaura, a significant portion of the AHSs appears to be aquagenic, derived from algal material. Fukushima et al. (1996), using the ultraviolet absorbance to DOC ratio, found that about 55% of the DOC at the center of Lake Kasumigaura was autochthonous.

Fulvic acid dominated over humic acid as AHSs in Lake Kasumigaura. This is consistent with previous reports. About 90% of the dissolved humic substances in uncolored surface waters occur as fulvic acid and only 10% or less as humic acid (Malcolm 1990). The focus should therefore be on fulvic acid rather than on humic acid when conducting research related to AHSs in lake waters.



© 1999 NRC Canada

Microcystis aeruginosa isolated from an algal bloom in Lake Kasumigaura produced hydroxamate-type siderophores under conditions of iron limitation. The growth pattern of *M. aeruginosa* differed from that of traditional nutrient-limited growth and was influenced by the induction of a high-affinity iron transport system. This was reflected by a precipitous increase in siderophore production, a slight recovery in μ, and a substantial increase in stationary Chl *a* concentration as the FHFe was decreased. However, there was not the observed substantial recovery of μ (greater than 80% of the μ, under iron-replete conditions) reported for other cyanobacteria by Brown and Trick (1992) and Wilhelm and Trick (1995).

Murphy et al. (1976) suggested that hydroxamate chelators produced by cyanobacteria may antagonize growth of other microalgae and hence be a factor in cyanobacterial blooms. However, this may not be true for *M. aeruginosa*. The hydroxamate-type siderophores produced by *M. aeruginosa* may not be as effective as those of other cyanobacteria when it comes to eliminating competing algal species in freshwater lakes by sequestering all the available Fe(III). This suggestion is consistent with the observation reported by McKnight and Morel (1979) that *M. aeruginosa* produces weakly binding hydroxamate-type siderophores. As Hutchins et al. (1991) proposed, the siderophores produced by *M. aeruginosa* may be most useful in helping the cyanobacterium maintain high biomass in quiescent water through recycling Fe(III) within the population, rather than promoting cyanobacterial dominance through scavenging and denying Fe(III) to other algae.

Fulvic acid isolated from Lake Kasumigaura exhibited a significant negative effect on the growth of *M. aeruginosa* under both iron-limited and iron-replete conditions. The fulvic acid concentration influenced the extent of the inhibition. Fulvic acid at 10 mg DW·L^{-1} completely inhibited the growth of *M. aeruginosa* over the pFHFe range studied. This contrasts with the growth patterns when fulvic acid was added at 2 mg DW·L^{-1}. Except for the lowest FHFe (pFHFe 12.9) in which no growth was observed, *M. aeruginosa* exhibited growth at all FHFes. However, the Chl *a* concentration abruptly declined during the exponential growth phase. The relatively abrupt transition from the exponential phase to the stationary phase and to the senescent phase suggests that a nutrient deficiency could explain this observation (Serrano and Guisande 1990). The patterns of the growth curves were similar to that observed without the addition of the fulvic acid at pFHFe 13.9, where the supply of Fe(III) was insufficient despite siderophore production being actively engaged (Fig. 2). Moreover, siderophore production was observed in the cell-free filtrates from culture media of pFHFes of 7.4, 9.9, 10.9, and 11.9. Therefore, the deficient nutrient is likely to be iron. We suggest that the fulvic acid binds so tightly to Fe(III) that the hydroxamate-type siderophores produced by *M. aeruginosa* cannot scavenge Fe(III) from the iron – fulvic acid complexes.

Several studies support this interpretation. Jackson and Hecky (1980) studied lakes and reservoirs in a boreal forest and reported an inverse correlation between primary productivity and humic, colloidal, hydrated ferric oxide complexes. They hypothesized that humic matter binds iron, making it unavailable to algae. Giesy (1976), studying the effect of humic acids on the growth of *Scenedesmus obliquus*, reported that humic acids decrease iron availability to iron-starved *S. obliquus* by tightly binding iron. Guildford et al. (1987), in an enrichment study using limnocorrals, observed that organic moss–peat materials lowered primary productivity and biomass and suggested that this was due to the binding of iron or some other metal by dissolved humic material. Our results are consistent with these observations.

To evaluate the extent of the affinity of AHSs toward Fe(III), the conditional stability constant between the fulvic acid and Fe(III) was estimated. The following assumptions were made for simplicity: (*i*) the fulvic acid is completely deprotonated, (*ii*) the Fe(III) – fulvic acid reaction is at equilibrium, and (*iii*) the complexation of the fulvic acid with other metals is negligible. The calculation with MINEQL was made for pFHFe 11.9 with the addition of fulvic acid at 2 mg·DW·L^{-1}. Under these conditions, the growth of *M. aeruginosa* was greatly inhibited and almost no growth was observed, which is similar to what was observed at pFHFe 13.9 in the absence of the fulvic acid. With fulvic acid at 2 mg·DW·L^{-1}, significant growth was observed at pFHFe 10.9, but no growth was observed at pFHFe 12.9. Therefore, pFHFe 11.9 with fulvic acid at 2 mg DW·L^{-1} may be regarded as an FHFe boundary below which the fulvic acid may complex tightly with Fe(III) so that the FHFe concentration would be below $10^{-13.9}$ M and *M. aeruginosa* cannot grow even with active siderophore production. The average molecular weight of the fulvic acid used is around 800 g·mol^{-1} (A. Imai, unpublished data); thus, fulvic acid at 2 mg DW·L^{-1} is about 2.5×10^{-6} M. The MINEQL calculation on the Fe(III) speciation in the medium indicated that the fulvic acid needs to have a conditional stability constant ($K'_{FeL} = [FeL]/[Fe^{3+}][L]$) for Fe^{3+} of about 10^{25}·M^{-1} to reduce the FHFe concentration below $10^{-13.9}$ M. This estimated stability constant value is extremely high, indicating that almost all ferric iron occurs organically complexed. This is consistent with findings reported in several recent studies on Fe(III) speciation in seawater: (*i*) the stability of natural organic complexes in seawater increases strongly in the order zinc < lead < copper << cobalt < nickel << iron, (*ii*) more than 99% of dissolved Fe(III) is strongly complexed by natural organic ligands, and (*iii*) the conditional stability constant K'_{FeL} is about 10^{20}–10^{23}·M^{-1} (van den Berg 1995).

Now we extend our discussion to the growth of *M. aeruginosa* in Lake Kasumigaura. Note that the carbon content of the fulvic acid used in this study was 54.0%, and thus, fulvic acid at 2 mg DW·L^{-1} was equivalent to 1.1 mg C·L^{-1}. Considering that the great majority of the AHSs in Lake Kasumigaura is fulvic acid, the fulvic acid at 2 mg DW·L^{-1} is almost the same as the average fulvic acid concentration found in Lake Kasumigaura (Fig.1A). The dissolved Fe(III) concentration in the surface water in the center of Lake Kasumigaura was about 10^{-7} M and remained relatively constant, showing no clear seasonal trend (Inaba et al. 1997). The measured pH was around 7.2 –9.6 from summer to fall. The MINEQL calculation with 2.5×10^{-6} M fulvic acid indicated that virtually all the Fe(III) exists in an organic complex form with fulvic acid and that the FHFe concentration shows a tendency to increase with increasing pH: $10^{-17.3}$, $10^{-14.0}$, $10^{-12.0}$, and $10^{-8.1}$ M at pH 7, 8.5, 9, and

© 1999 NRC Canada

10, respectively. Thus, *M. aeruginosa* may not be able to grow at pH lower than 8.5 and may be under significant iron limitation at pH greater than 8.5. Hence, it can be proposed that *M. aeruginosa* in Lake Kasumigaura should be severely iron limited because of AHS–Fe(III) complexation.

In eutrophic Lake Kasumigaura, heavy blooms of *Microcystis* spp. (mostly *M. aeruginosa*) were observed until 1986. They suddenly disappeared in 1987. Since then, *Microcystis* has declined in number, and instead, filamentous cyanobacteria such as *Oscillatoria* spp. (*Oscillatoria tenuis* and *O. agardhii*) have become dominant in summer and fall (Takamura et al. 1992; Yagi et al. 1995).

Yagi et al. (1987) conducted algal growth potential tests for filtered waters of Lake Kasumigaura in 1981 when *Microcystis* algal blooms were frequently observed. They found that the growth of *M. aeruginosa* in the filtrates was stimulated by phosphorus and nitrogen in summer but by inorganic iron ($FeCl_3$) or EDTA from fall to spring and recognized the importance of iron as a limiting nutrient for *M. aeruginosa*.

Subsequently, Yagi et al. (1995) conducted a similar study from April to October 1992, when *Microcystis* blooms had already disappeared. They found that *M. aeruginosa* did not grow at all in lake filtrates when nitrogen and phosphorus were supplemented. The growth of *M. aeruginosa* in the filtrates was stimulated by the addition of EDTA in August and by the additions of EDTA and phosphorus over the rest of the year. Moreover, the addition of inorganic iron no longer stimulated growth. These findings clearly indicate that the characteristics of the water in Lake Kasumigaura had significantly changed sometime between 1981 and 1992.

Without the addition of EDTA, *M. aeruginosa* could not grow in the lake water filtrates from Lake Kasumigaura. It seems unlikely that the stimulation by EDTA resulted from reduction in the toxicity of metals such as copper (Inaba et al. 1997). Since EDTA may make essential trace metals more available to phytoplankton, one possibility is that iron may be a limiting nutrient for *M. aeruginosa*. The iron available to *M. aeruginosa* may have become insufficient after 1987. Taking into consideration the significant inhibition, probably by iron limitation, caused by the fulvic acid of the growth of *M. aeruginosa* observed in this study, we suggest that the AHSs may be responsible for the recent disappearance of *M. aeruginosa* from Lake Kasumigaura and that the concentration of AHSs in the lake water may be increasing.

It is interesting to note that *O. tenuis*, different from other cyanobacteira, produces multiple siderophores: both tradiotional hydroxamate-type and catechol-type siderophores (Brown and Trick 1992). Catechol-type siderphores have 5–13 orders higher affinity for iron as compared with hydroxamate-type ones (Lewis et al. 1995). *Oscillatoria* spp. may be able to survive in Lake Kasumigaura by scavenging iron from Fe(III)–AHS complexes.

In this study, we have shown that in eutrophic Lake Kasumigaura, AHSs were around 1 mg $C \cdot L^{-1}$ and accounted for 16–35% of the DOC. We have also shown that fulvic acid dominated over humic acid, accounting for more than 85% of the AHSs. Laboratory algal growth experiments, using *M. aeruginosa* isolated from an algal bloom in Lake Kasumigaura and fulvic acid isolated from the same lake water, confirmed explicitly that the ambient level of the fulvic acid in Lake Kasumigaura significantly inhibited the growth of *M. aeruginosa*, probably through Fe(III) – fulvic acid complexation, although *M. aeruginosa* did produce hydroxamate-type siderophores under iron-limited conditions. AHSs may play an important role in the formation of algal blooms of *M. aeruginosa* and in changes in cyanobacterial species composition in eutrophic lakes.

Acknowledgements

We thank M. Aizaki, K. Kohata, and Y. Nakamura for helpful and insightful discussion.

References

Anderson, M.A.,and Morel, F.M.M. 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*. Limnol. Oceanogr. **25**: 789–813.

Boyer, G.L., Gillam, A.H., and Trick, C.G. 1987. Iron chelation and uptake. *In* The cyanobacteria. *Edited by* C. Van Baalen and P. Fay. Elsevier Science, New York. pp. 415–436.

Brand, L.E. 1991. Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production. Limnol. Oceanogr. **36**: 1756–1771.

Brown, C.M., and Trick, C.G. 1992. Response of the cyanobacterium, *Oscillatoria tenuis*, to low iron environments: the effect on growth rate and evidence for siderophore production. Arch. Microbiol. **157**: 349–354.

Fukushima, T. (*Editor*). 1995. Proceedings of the Conference on Limnological Studies at the Kasumigaura Water Research Station. Part 9. Natl. Inst. Environ. Stud. F-76-'95/NIES. National Institute for Environmental Studies, Ibaraki, Japan. (In Japanese.)

Fukushima, T., Park, J., Imai, A., and Matsushige, K. 1996. Dissolved organic carbon in a eutrophic lake; dynamics, biodegradability and origin. Aquat. Sci. **58**: 139–157.

Giesy, J.P., Jr. 1976. Stimulation of growth in *Scenedesumus obliquus* (Chlorophyceae) by humic acids under iron limited conditions. J. Phycol. **12**: 172–179.

Gillam, A.H., Lewis, A.G., and Anderson, R.J. 1981. Quantitative determination of hydroxamic acids. Anal. Chem. **53**: 841–844.

Guildford, S.J., Healey, F.P., and Hecky, R.E. 1987. Depression of primary production by humic matter and suspended sediment in limnocorral experiments at Southern Indian Lake, Northern Manitoba. Can. J. Fish. Aquat. Sci. **44**: 1408–1417.

Hudson, R.J.M., Convault, D.T., and Morel, F.M.M. 1992. Investigations of iron coordination and redox reactions in seawater using ^{59}Fe radiometry and ion-pair solvent extraction of amphiphilic iron complexes. Mar. Chem. **38**: 209–235.

Hutchins, D.A., Rueter, J.G., and Fish, W. 1991. Siderophore production and nitrogen fixation are mutually exclusive strategies in *Anabaena* 7120. Limnol. Oceanogr. **36**: 1–12.

Inaba, K., Sekine, T., Tomioka, N., and Yagi, O. 1997. Seasonal and longitudinal changes in copper and iron in surface water of shallow eutrophic Lake Kasumigaura, Japan. Water Res. **31**: 280–286.

Ingle, R.M., and Martin, D.F. 1971. Prediction of the Florida red tide by means of the iron index. Environ. Lett. **1**: 69–74.

Jackson, T.A., and Hecky, R.E. 1980. Depression of primary productivity by humic matter in lake and reservoir waters of the boreal forest zone. Can. J. Fish. Aquat. Sci. **37**: 2300–2317.

Kerndorff, H., and Schnizer, M. 1980. Sorption of metals on humic acid. Geochim. Cosmochim. Acta, **44**: 1701–1708.

© 1999 NRC Canada

Leenher, J.A. 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. Environ. Sci. Technol. **15**: 578–587.

Lewis, B.L., Holt, P.D., Taylor, S.W., Wilhelm, S.W., Trick, C.G., Butler, A., and Luther, G.W., III. 1995. Voltammetric estimation of iron(III) thermodynamic stability constants for cathecholate siderophores isolated from marine bacteria and cyanobacteria. Mar. Chem. **50**: 179–188.

Malcolm, R.M. 1990. The uniqueness of humic substances in each of soil, stream and marine environments. Anal. Chim. Acta, **232**: 19–30.

McKnight, D.M., and Morel, F.M.M. 1979. Release of weak and strong copper-complexing agents by algae. Limnol. Oceanogr. **24**: 823–837.

McKnight, D.M., Andrews, E.D., Spaulding, S.A., and Aiken, G.R. 1994. Aquatic fulvic acids in algal-rich Antarctic ponds. Limnol. Oceanogr. **39**: 1972–1979.

Morel, F.M.M., Rueter, J.G., Jr., Anderson, D.M., and Guillard, R.R.L. 1979. AQUIL: a chemically defined phytoplankton culture medium for trace metal studies. J. Phycol. **15**: 135–141.

Murphy, T.P., Lean, D.R.S., and Nalewajko, C. 1976. Blue-green algae: their excretion of iron-selective chelators enables them to dominate other algae. Science (Washington, D.C.), **192**: 900–902.

Neilands, J.B. 1981. Microbial iron compounds. Annu. Rev. Biochem. **50**: 715–731.

Prakash, A., Rashid, M.A., Jensen, A., and Subba Rao, D.V. 1973. Influence of humic substances on the growth of marine phytoplankton: diatoms. Limnol. Oceanogr. **18**: 516–524.

Serrano, L., and Guisande, C. 1990. Effects of polyphenolic compounds on phytoplankton. Verh. Int. Ver. Limnol. **24**: 282–288.

Smith, R.M., and Martel, A.E. 1975. Critical stability constants. Vol. 2. Amino acid. Plenum Press, New York.

Stumm, W., and Morgan, J. 1981. Aquatic chemistry. 2nd ed. John Wiley & Sons, New York.

Takamura, N., Otsuki, A., Aizaki, M., and Nojiri, Y. 1992. Phytoplankton species shift accompanied by transition from nitrogen dependence to phosphorus dependence of primary production in Lake Kasumigaura, Japan. Arch. Hydrobiol. **124**: 129–148.

Thurman, E.M. 1985. Organic geochemistry of natural waters. Martinus Nijhoff/W. Junk Publishers, Dordrecht, The Netherlands.

Thurman, E.M., and Malcolm, R.L. 1981. Preparative isolation of aquatic humic substances. Environ. Sci. Technol. **15**: 463–466.

Tipping, E., and Woof, C. 1983. Seasonal variations in the concentrations of humic substances in a soft-water lake. Limnol. Oceanogr. **28**: 168–172.

van den Berg, C.M.G. 1995. Evidence for organic complexation of iron in seawater. Mar. Chem. **50**: 139–157.

Watanabe, M.W., and Nozaki, H. 1994. NIES-collection, list of strains: microalgae and Protozoa. 4th ed. Natl. Inst. Environ. Stud. F-60-'93/NIES. National Institute for Environmental Studies, Ibaraki, Japan.

Westall, J.C., Zachary, J.L., and Morel, F.M.M. 1976. MINEQL: a computer program for the calculation of chemical equilibrium composition of aqueous systems. MIT R.M. Parsons Lab. Tech. Note No. 18. Massachusetts Institute of Technology, Cambridge, Mass.

Wilhelm, S.W., and Trick, C.G. 1995. Physiological profiles of *Synechococcus* (Cynaophyceae) in iron-limiting continuous cultures. J. Phycol. **31**: 79–85.

Yagi, O., Hagiwara, T., Takamura, Y., and Sudo, R. 1987. Limiting nutrients of algal growth in Lake Kasumigaura. Jpn. J. Water Pollut. Res. **10**: 115–122. (In Japanese with English abstract.)

Yagi, O., Uchiyama, H., Tomioka, N., Sakurai, M., and Takamura, Y. 1995. Limiting nutrients of several blue-green algal species in Lake Kasumigaura. *In* Aoko (water-blooms of blue-green algae); measurement, occurrence, and factors on its growth. *Edited by* T. Fukushima and M. Aizaki. Natl. Inst. Environ. Stud. F-72-'95/NIES. National Institute for Environmental Studies, Ibaraki, Japan. pp. 51–61. (In Japanese.)

© 1999 NRC Canada

The role of copepod-dominated meiofauna in the mineralization of organic matter in a cold marine mesocosm

Serge Parent and Antoine Morin

Abstract: Large populations of meiofauna are found in the sand filters of the St. Lawrence mesocosm at the Montreal Biodome. Three 30-day experiments were conducted in heterotrophic microcosms to quantify how populations of micro- and meio-faunal organisms affect mineralization using the apparent mineralization rate (AMR), i.e., nitrate production, as a proxy. Tryptone, mesocosm detritus, and fishmeal were used as organic substrates (C:N ratios 4–8). Harpacticoid copepods dominated the meiofauna in numbers (87%) and biomass (90%). AMR was inversely related to meiofaunal mass and not related to ciliate density. Through grazing, 1 g meiofaunal dry mass·m^{-2} reduced the AMR of tryptone by 42%, of detritus by 9.4%, and of fishmeal by 2.7%. Particle size affected the effect of meiofauna, whereas the C:N ratio affected the AMR. The scarcity of nematodes, which are known to stimulate mineralization, may explain these results. Copepod-dominated meiofauna decrease nutrient regeneration rates in heterotrophic habitats when C:N ratios are low.

Résumé : Les filtres à sable du mésocosme du Saint-Laurent marin du Biodôme de Montréal renferment d'importantes populations de méiofaune. Trois expériences de 30 jours furent menées dans des microcosmes hétérotrophiques pour mesurer l'effet de cette méiofaune et de la microfaune sur la minéralisation en utilisant le taux apparent de minéralisation (TAM), c.-à-d., la production des nitrates, comme indicateur. Du tryptone, des détritus du mésocosme et de la farine de poisson furent utilisés comme substrats organiques (rapport C:N 4–8). Les copépodes harpacticoïdes dominent la méiofaune en nombre (87%) et en biomasse (90%). Le TAM fut inversement corrélé à la masse de méiofaune mais non à la densité des ciliés. Par le broutage, 1 g masse sèche·m^{-2} de méiofaune réduit le TAM du tryptone de 42%, celui des détritus de 9.4% et celui de la farine de poisson de 2.7%. La taille des particules a affecté l'effet de la méiofaune tandis que le rapport C:N a affecté le TAM. La rareté des nématodes, connus pour stimuler la minéralisation, explique peut-être ces résultats. Une méiofaune dominée par les copépodes diminue le taux de régénération des nutriments dans les habitats hétérotrophiques lorsque le rapport C:N est faible.

Introduction

The mineralization of organic matter is an important process in all aquatic habitats, but is of utmost concern in closed systems such as aquaria and mesocosms. In these aquatic systems, impairment of the mineralization process, i.e., dead fish or accumulation of detritus, generally results in a build-up of ammonia, the toxicity of which leads to the death of the fish and invertebrates on display. In a healthy system, ammonia produced by the digestion of the organic material by heterotrophic bacteria is quickly nitrified into much less toxic nitrate by chemoautotrophic bacteria. Biological filtration systems for large marine aquaria, such as the St. Lawrence mesocosm of the Montreal Biodome, depend on this bacterial activity to maintain life support.

The rate at which organic matter is mineralized into ammonia is directly related to bacterial biomass and metabolic rate. However, it is also affected by animals that graze these bacteria, bioturbate sediments, or fragment organic matter. Direct and indirect evidence suggest that marine meiofauna increases the mineralization rate of organic matter. Direct evidence comes mainly from short-term laboratory experiments done with nematodes (Tenore et al. 1977; Findlay and Tenore 1982; Alkemade et al. 1992). Indirect evidence is provided by reports that nematodes and copepods increase bacterial biomass (and presumably mineralization) through bactericulture (Heip et al. 1985; Hicks and Coull 1983), symbiosis (Heip et al. 1985), bioturbation (Aller and Aller 1992), fragmentation (Tenore et al. 1977), and even through grazing (Montagna 1984; Epstein and Shiaris 1992). Grazing by meiofauna eliminates senescent cells and maintains colonies in a state of exponential growth (Yingst and Rhoads 1980; Meyer-Reil and Faubel 1980) and therefore can lead to an increase of bacterial densities.

The present study was carried out to test whether the meiofauna living in the sand filters of the St. Lawrence mesocosm (SLM) positively affect mineralization. Experi-

Received September 18, 1998. Accepted April 27, 1999. J14790

S. Parent[1] **and A. Morin.** Ottawa-Carleton Institute of Biology, Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, ON K1N 6N5, Canada.

[1]Author to whom all correspondence should be sent at the following address: Biodôme de Montréal, 4777 Pierre-De Coubertin, Montréal, QC H1V 1B3, Canada. e-mail: sergeparent@ville.montreal.qc.ca

© 1999 NRC Canada

Fig. 1. Hydraulic diagram of the St. Lawrence mesocosm: MP, main pool, medical pool, and tidepool; RSF, sand filters (F1–F6); TF, trickling filter; OT, ozone tower; HE, heat exchangers.

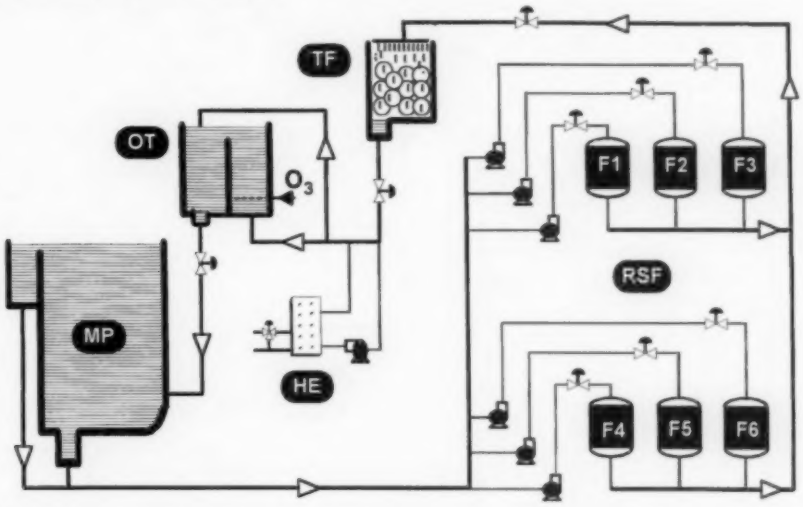


Table 1. Particle size, content in organic matter, organic nitrogen and organic carbon, and C:N ratio of the detritus in sand filters and of the three organic substrates added to the microcosms (mean ± SE).

	Particle size, µm	Organic content, $g \cdot m^{-2}$	Nitrogen content, $g\ N \cdot m^{-2}$	Carbon content, $g\ C \cdot m^{-2}$	C:N ratio
Sand filters	400	295±18.7	5.9±0.31	31.9±3.66	6.3±0.18
Microcosms					
Tryptone, 4 g	<0.45	51	4.5±0.01	17.1±0.03	4.4±0.08
Detritus, 22 g	400	281	6.0±0.01	40.9±0.05	7.9±0.09
Fishmeal, 5 g	100–150	64	7.4±0.02	29.4±0.18	4.6±0.11

ments were conducted in laboratory model systems (heterotrophic microcosms) containing synthetic seawater and fixed quantities of three organic substrates differing in particle size and C:N ratios. Inhibitors specific to eukaryotic cells were used to selectively control microfaunal and meiofaunal abundances. Nitrate production was measured as a proxy for mineralization because ammonia, the end product of mineralization, is transient and quickly transformed into nitrate by the nitrification process. Because the rate of nitrate production is a composite of the mineralization rate (R-$NH_2 \rightarrow NH_3$), the nitrification rate ($NH_3 \rightarrow NO_3$), and the faunal metabolic rates ($NH_3 \leftrightarrow R$-NH_2) within the system, the experiments produced apparent mineralization rate (AMR) (see Parker et al. 1989). Nitrate concentrations were measured at regular time intervals to estimate the AMR, which were then correlated with protozoan and meiofaunal abundances.

Materials and methods

The St. Lawrence mesocosm

The St. Lawrence marine mesocosm is a closed system that holds 3×10^6 L of cold (10°C) artificial seawater (28–30‰). It exhibits fish, invertebrates, and seabirds from the Gulf of St. Lawrence. The water treatment system consists of six sand filters, a 200 m^3 trickling filter filled with plastic bioballs, and an ozonation system (Fig. 1). The sand filters are 3.6 m in diameter and contain 65 cm of fine sand (250 µm to 300 µm effective particle size). The water rate through the filters is 6 $L \cdot m^{-2} \cdot s^{-1}$. The filters are backwashed approximately every 5 days. All detritus ends up in the sand filters, where most of the meiofauna is located (S. Parent, unpublished data). Pre-test analyses showed that the top 2.5 cm of sand contains 276–314 g organic matter dry mass$\cdot m^{-2}$ before backwash (Table 1). The organic matter contains by weight about 2% nitrogen (N) and has a C:N ratio of 6.1–6.5. Water temperature is maintained throughout the year at 10°C. Nitrate levels in the mesocosm water were above 100 mg NO_3-$N \cdot L^{-1}$ at the time of the experiments.

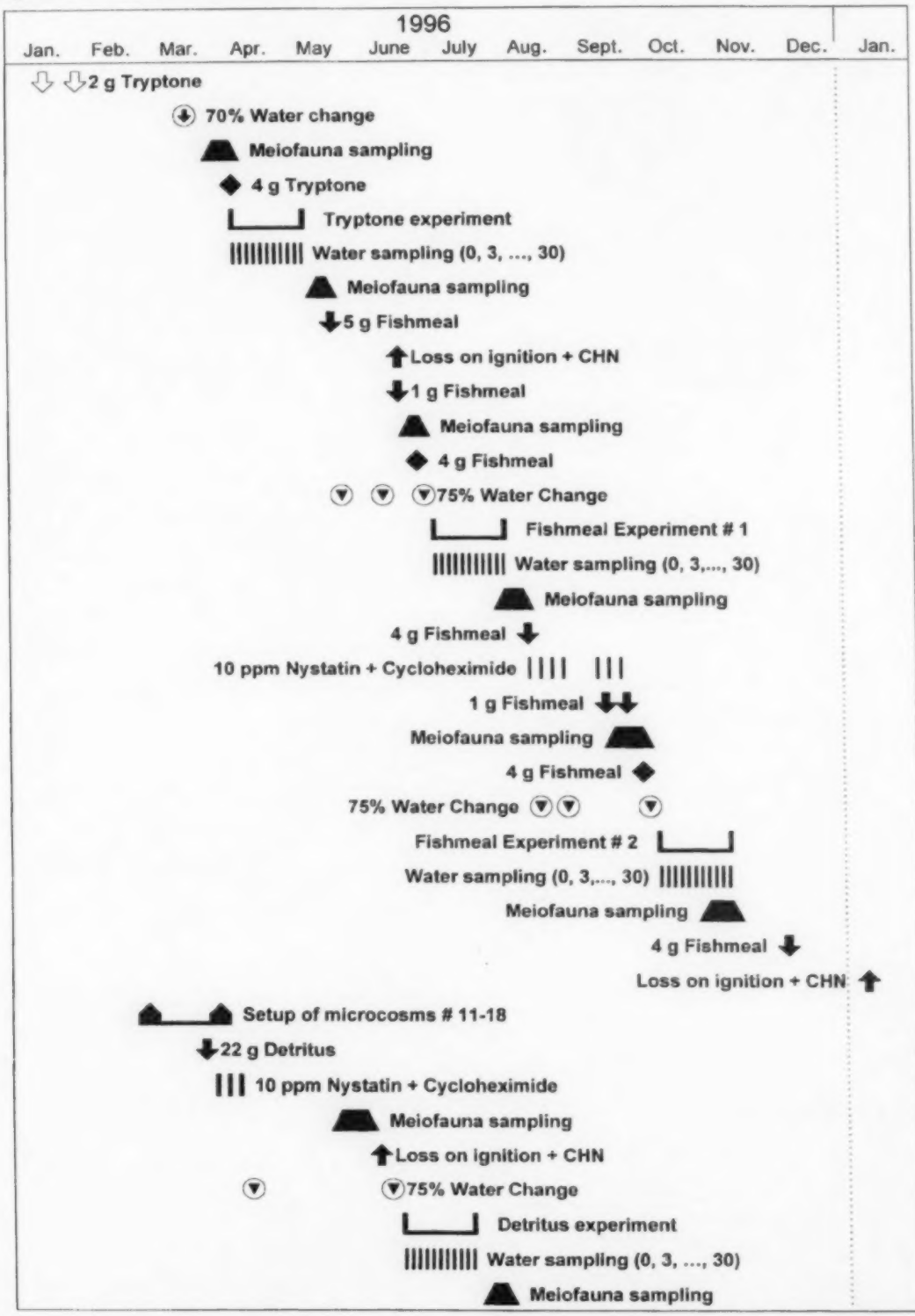
Experimental setup

Three experiments were conducted under controlled conditions using tryptone, mesocosm detritus from the sand filters, and fishmeal as organic subtrates. Tryptone and fishmeal, differing from detritus in particle size or C:N ratio, were used to test whether these factors influenced meiofaunal effects on the mineralization rate of detritus.

Two sets of eight heterotrophic microcosms were used to conduct the experiments. Each microcosm consisted of a 20-L aquarium (778 cm^2) containing 13.5 L of water and an undergravel filter with two airlifts (filtration rate 0.7–1.4 $L \cdot m^{-2} \cdot s^{-1}$, turnover rate of 2–4 min). The bottom of each microcosm was covered with 4 cm of filter sand. A shallow sand bed and a uniform yet rapid flow of oxygen-saturated water from top to bottom of the sand bed insured constant oxygen saturation in the sediment interstitial water and prevented the formation of anoxic zones. Seawater (28–30‰) was prepared using Instant Ocean® salts. The microcosms were maintained at 10°C in the dark to prevent algal growth.

© 1999 NRC Canada

Fig. 2. Gantt chart of the three experiments. The tryptone and fishmeal experiments were done using microcosms 1–8, whereas the detritus experiment was done using microcosms 11–18.



© 1999 NRC Canada

Table 2. Relative abundances (individuals·10^3 specimens) of meiofauna in the experimental microcosms and sand filters of the St. Lawrence mesocosm.

Taxon	Microcosms	Sand filters
Copepoda harpacticoida	871	911
Pseudonychocamptus proximus		
Tisbe (*furcata*?)		
Paramphisciella (*fulvofasciata*?)		
Nematoda	81	76
Anticoma sp.		
Chomadoridae sp.		
Monhystera sp.		
Rotifera	35	7
Colurella (*colurus*?)		
Dicranophoridae sp.		
Turbellaria	3.5	2.3
Species A		
Species B		
Species C		
Species D		
Polychaeta	5	0.4
Dinophilus (*gyrociliatus*?)		
Nerilla sp.		
Gastrotricha	4	2.0
Chaetonotida sp.		
Acarina	1	0.3
Thalassarachna basteri		
Isobactrus setosus		
Copidognathus biodomus nov. sp.		
Hydrozoa	—	1
Corynidae sp.		
Clavidae sp.		
Pandeidae sp.		
Hydractinia echinata		

The experiment with tryptone was run in April 1996 (day 0 = start of the experiment) with one set of microcosms (Fig. 2). Eight months before (day –250), meiofauna were extracted from the sand of one of the mesocosm filters by decantation (Pfannkuche and Thiel 1988) and put in the microcosms. Meiofauna were then maintained on tryptone until day 0. On day –204, meiofauna were suppressed in three microcosms with one dose of 10 ppm nystatin and cycloheximide (Sigma Chemical Co., St. Louis, Mo., U.S.A. 63178), two substances inhibitory to eucaryotic organisms (Lee and Welander 1994). On day –20, 70% of the water was replaced, and on day –1, water levels were adjusted. On day 0, 4 g of tryptone was quickly dissolved in each microcosm. Nitrate levels on day 0 were between 13.7 and 17.5 mg NO_3-N·L^{-1}.

The tryptone used was soybean-casein digest broth (Difco 1984). When dissolved, it had a particle size less than 0.45 µm. It contained 8.7% nitrogen and had a C:N ratio of 4.4 (Table 1). By adding 4 g of tryptone to each tank, we were adding about the same amount of nitrogen (4.5 g N·m^{-2}) but less carbon (17.1 g C·m^{-2}) than what is found in the upper 2.5-cm layer of a sand filter. Given the small amounts of tryptone added before the experiment, there was probably little organic nitrogen in the sand on day 0.

The experiment with detritus was run in June 1996 with the other set of eight microcosms (Fig. 2). These were set up 87 days before the start of the experiment (day –87). A mesocosm sand filter clogged with detritus was accessed and 4 cm of wet sand was put in each microcosm. From day –82 to day –68, meiofauna were suppressed in four microcosms using repeated dosages of 10 ppm nystatin and cycloheximide. On day –2, 75% of the water was replaced. Nitrate levels on day 0 were between 26 and 35 mg NO_3-N·L^{-1}. All microcosms had organic matter, N, and C contents within the range of values in the sand filters (Table 1). On day –17, the organic matter content of the sand was 281 g·m^{-2}. On day –2, the organic matter contained 2.2% nitrogen and had a C:N ratio of 7.9.

To determine particle size, detritus was collected in a sand filter, dried at 60–80°C, and put through a geometric series of test sieves with 1.0 phi (Ø) intervals. The median particle diameter was 400 µm (Table 1). Over 35% of the particles were larger than 1 mm; 4.4% were less than 300 µm.

The eight microcosms used for the tryptone experiment were used again for the fishmeal experiment and were fed fishmeal in May 1996 (Fig. 2). One trial was done in July, and another in October 1996. Before the start of each trial (day –44), meiofauna was depressed in 2 or 3 microcosms using repeated dosages of 10 ppm nystatin and cycloheximide. This caused the loss of three microcosms in the second trial. Fishmeal (1 g) was added to each microcosm on day –14 and 4 g more were added on day –7. On day –3, 75% of the water was changed. Nitrate levels on day 0 were between 15 and 24 mg NO_3-N·L^{-1}.

Redfish (*Sebastes marinus*) fishmeal, produced locally, was used in the experiments. It contained 11.5% nitrogen and had a C:N ratio of 4.6 (Table 1). The fishmeal particle size was between 100 and 150 µm. Before adding fishmeal, each microcosm contained about 0.6 g N·m^{-2} and 6.3 g C·m^{-2}. Through the addition of 5 g of fishmeal, each microcosm contained the same amount of N and C as the upper 2.5-cm layer of a sand filter (Table 1).

Water sampling in all experiments began on day 0 and occurred every 3 days for 30 days (Fig. 2). Four water samples (28 mL total) were taken from each microcosm and quickly frozen at –20°C. Water losses through sampling and evaporation amounted to 5–10% during the experiments. No water was added during the experiment and no corrections for water loss were made in data analyses.

Biological determinations

Microfauna and meiofauna were generally measured no more than 10 days before and after each experiment. Because meiofauna are patchy in their distribution (Findlay 1981), the sand was combed before each sampling to obtain a better estimate of the meiofaunal abundance with the least effort. Five to 10 samples of the 0- to 2.5-cm layer were randomly taken with a 10-cm^3 syringe (1.63 cm^2). Meiofauna and microfauna were separated in a 50-cm^3 cylinder using the decantation method (Pfannkuche and Thiel 1988) and then collected on a 20-µm sieve. By closely examining 1 cm^3 of residue from randomly chosen samples, decantation was estimated to be at least 95% effective in extracting meiofauna. All animals were counted live. A different set of samples was taken to assess the standard weight of each taxon: length and width of specimens were measured and then converted into weights using conversion factors (Feller and Warwick 1988). These standard weights were then combined with the abundance results to establish the meiofaunal mass of each sample. The sand of all samples was put back into the microcosms but not the meiofauna. Sampling removed an insignificant amount of meiofauna (between 1 and 2%) before each experiment.

Three groups of protozoans were observed: ciliates, foraminiferans, and heliozoans. Only ciliates were quantified. Ciliate populations were found by Fenchel (1978) to outnumber all other fauna in well-mixed (100–300 µm) medium sands with little interstitial silt and clay, as in the case here. They were therefore used as an index of protozoan abundance.

Chemical analysis

Nitrate was measured on a Tecator FIAstar, model 5010, using the automated cadmium reduction method (APHA et al. 1992) and

© 1999 NRC Canada

the analysis protocol for 2–200 mg NO_3-N·L^{-1}. AMR results were then converted to mg NO_3-N·m^{-2}·day^{-1} using the water volume and sand surface of each microcosm. Carbon and nitrogen analyses were performed on a Carlo-Erba CHN analyzer. Organic matter content was determined by loss on ignition at 400–450°C.

Statistical analysis

The relationships between AMR and microfaunal number (individuals·10 cm^{-2}) or meiofaunal biomass (g dry mass·m^{-2}) were quantified by linear regression analysis. For each microcosm, the biomass was set as the arithmetic mean of the biomasses before and after the experiment. Regression models for each of the three organic substrates were compared by covariance analysis. Detailed analysis of mineralization was done by looking at the AMR_t, i.e., AMR on days t = 6, 9, 12, ..., 24, during each experiment. AMR_t of each microcosm was calculated by regressing five nitrate concentrations (t–6, t–3, t, t+3, and t+6) over time. Each set of AMR_t was then regressed against meiofaunal biomass. The resulting Y-intercept (AMR_0), regression coefficient (effect of meiofauna) and R^2 of each regression were plotted against time to describe the phases of each experiment.

Results

Meiofaunal composition and abundance

Meiofauna was comprised of seven taxa: harpacticoid copepods, nematodes, rotifers, polychaetes, turbellarians, mites (Acari), and gastrotrichs (Table 2). The harpacticoids dominated in both number (87%) and biomass (98%), with three species present (*Tisbe* (*furcata*?), *Pseudonychocamptus proximus*, and *Paramphiascella* (*fulvofasciata*?)). *Pseudonychocamptus* largely dominated the group with over 90% of the specimens. Nematodes and rotifers accounted for only 4.6% (8%) of the specimens and 0.8% (0.5%) of the biomass; other taxa represented 1% of the specimens and the biomass. The composition of the sand filter meiofauna was the same except for a few hydrozoan polyps absent in the microcosms.

Meiofaunal biomass in the 0- to 2.5-cm layer of the sand filters varied from 0.4 to 40 g dry mass·m^{-2} (1992–1996) with values of 20–40 g dry mass·m^{-2} during the year that the experiments took place (1996). Later sampling (July 1997) revealed that meiofauna were abundant throughout the sand column with values close to 1 g·m^{-2}, in core increments of 2.5 cm, at least down to 25 cm (S. Parent, unpublished data).

Meiofaunal abundance in the tryptone microcosms varied from 0 to 1.10 g dry mass·m^{-2}. One microcosm was excluded from analyses on the basis of unusually high mortality rate (50%) of the inoculated meiofauna. The mortality in that microcosm continued for at least 1 month after the experiment. Preliminary statistical analysis also revealed this microcosm to be an outlier (Studentized residual = 4.28).

Meiofaunal biomass in the detritus microcosms varied from 0 to 1.01 g dry mass·m^{-2}. Four microcosms had little meiofauna throughout, whereas in the four other microcosms, the meiofaunal biomass decreased at some point during the experiment from 1.5–1.8 to 0.13–0.24 g dry mass·m^{-2}.

Meiofaunal biomass in the fishmeal microcosms varied from 0.2 to 6.3 g dry mass·m^{-2}. We compared the meiofaunal biomasses of the five microcosms that were used for both trials and no correlation was found between them. This lack of correlation and the long interval between the two trials (98 days) imply that the two trials constituted 13 independent replicates of the experiment.

Apparent mineralization rate (AMR)

In the tryptone experiment, the production of nitrate during the 30-day period varied from 19 to 23 mg NO_3-N·L^{-1}. This represents 73–89% of the organic nitrogen present at the start. In contrast to the two other experiments, the production rate of nitrate during the experiment was clearly biphasic (Fig. 3). In all eight microcosms, there was a rapid increase in nitrates from day 0 to day 6, followed by a slow and steady production from day 6 to day 30. The AMR were measured for the 6- to 30-day period through linear regression. The standard error of the regression slopes was between 8% and 18% of the slope value.

In the detritus experiment, the production of nitrate during the 30-day period varied from 10 to 12 mg NO_3-N·L^{-1}. This represents 28.7–34.1% of the organic nitrogen present at the start. The production rate of nitrate was steady throughout the experiment (Fig. 3). The AMR could therefore be estimated by linear regression. The standard error of the regression slopes was between 5% and 20% of the slope value.

In the fishmeal experiment, the production of nitrates during the 30-day period of each trial varied from 12 to 16 mg NO_3-N·L^{-1}. This represents 27–36% of the organic nitrogen present at the start. The rate of nitrate production was slightly curvilinear during each trial (Fig. 3) but the AMR could still be estimated by linear regression with enough accuracy. The standard error of the regression slopes was between 6% and 11% of the slope value.

Water loss from sampling was constant at 0.7%, whereas water loss from evaporation varied from 4% to 9% from one experiment to another. Water loss was the same in all the microcosms of a given experiment; therefore, no correction was needed to estimate the effect of meiofauna on AMR although it resulted in slight overestimations of AMR.

Linear regression models

In all three experiments, the AMR was not related to ciliate density but was inversely related to meiofaunal biomass (Table 3). Ciliate densities were stable throughout the tryptone and fishmeal experiments but dropped from 210 000 individuals·m^{-2} to less than 15 000 individuals·cm^{-2} in the detritus experiment (Table 3). The ciliate density in the 0- to 2.5-cm layer of the sand filters was 192 000 ± 92 630 individuals·m^{-2} (mean ± SEM, n = 8).

There was a negative relationship between AMR and meiofaunal biomass (Fig. 4). In each experiment, the relationship was significant and meiofaunal biomass explained between 44% and 80% of the variation in AMR (Table 4). The effect of meiofaunal biomass varied among the organic substrates: 1 g meiofauna reduced the AMR of tryptone by 42%, that of mesocosm detritus by 9.4%, and that of fishmeal by 2.7% (Table 4).

Covariance analysis showed that the effects of meiofauna in the detritus and fishmeal experiments were similar ($\alpha F_{1,18}$ = 1.42, p = 0.25) but that the AMR_0 were different ($\alpha F_{1,18}$ = 99.8, $p < 0.001$). It also showed that the effects of meiofauna in the fishmeal and tryptone experiments were different ($\alpha F_{1,18}$ = 11.1, p = 0.004) and therefore the AMR_0

© 1999 NRC Canada

Fig. 3. Production of nitrate during the tryptone, detritus, and fishmeal experiments. Different symbols represent each of the microcosms in the experiment.

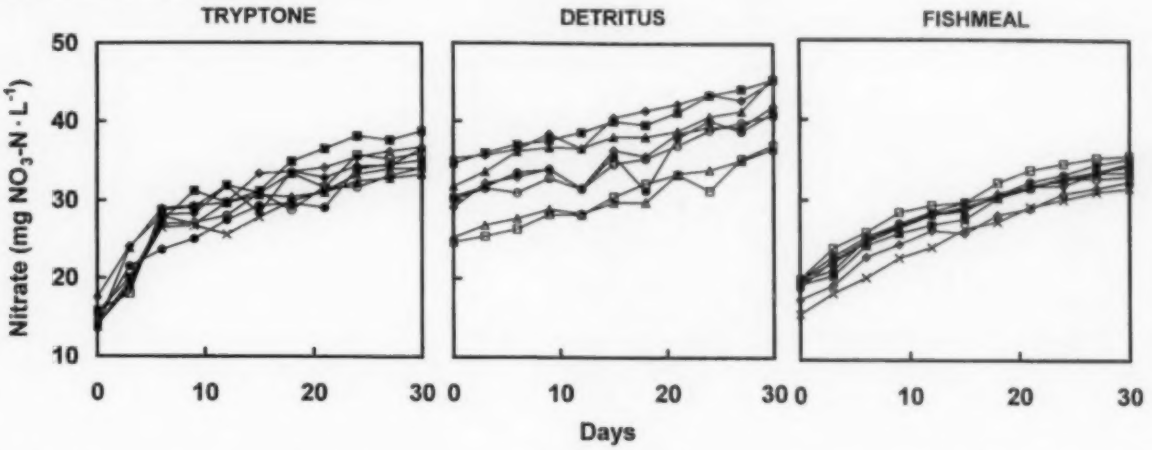


Table 3. Ciliate densities in the mineralization experiments and results of the Pearson correlations between AMR and ciliate

Experiment	Ciliate densities (individuals·m^{-2})	Ciliates		Meiofaunal mass	
		r	p	r	p
Tryptone	2500 – 42 000	–0.65	0.12	–0.89	0.007 **
Detritus	0 – 210 000	–0.34	0.40	–0.79	0.02 *
Fishmeal	5000 – 371 000	–0.43	0.14	–0.64	0.02 *

Note: * $0.01 < p \leq 0.05$; ** $p \leq 0.01$.

could not be tested. The detritus and tryptone models were not compared because of unequal residual variances.

Phases of the experiments

In the tryptone experiment, AMR_0, i.e., the AMR when there is no meiofauna, did not vary much and meiofauna always had a strong negative effect on AMR (Fig. 5). About 30% of the tryptone had been mineralized on day 12. The R^2 was above 0.50 on days 18 and 21. No data are available for days 6 and 9.

In the detritus experiment, AMR_0 increased until day 18 and then decreased slightly. Meiofauna had a positive effect on days 6 and 9. Its negative effect appeared strongly on day 12, was maintained through day 15, but was then quickly reduced to near 0 on days 18 and 21. On days 12 and 15, 12 to 15% of the detritus had been mineralized and the R^2 were above 0.20 (Fig. 5).

In the fishmeal experiment, AMR_0 decreased from 120 to less than 60 mg NO_3-N· m^{-2}·day^{-1} throughout the experiment. Meiofauna had no significant effect on day 6. Its negative effect appeared on day 9, increased until day 18, and then decreased. About 15 to 18% of the fishmeal had been mineralized by days 15 and 18. The R^2 was above 0.20 only on days 18 and 21 (Fig. 5).

AMR rates in all three experiments can be divided into three phases: an early phase (days 6 and 9), a middle phase (days 12,15, and 18), and a late phase (days 21 and 24). During the early phase, the effects of meiofauna were either null or slightly positive, the R^2 were less than 0.10, and the AMR_0 values were either low or high. During the middle phase, the effects of meiofauna became strongly negative, the R^2 reached values as high as 0.83, and AMR_0 values were high. Finally, during the late phase, the effects of meiofauna became less and less negative, the R^2 reached low values again, and the Y-intercepts (AMR_0) decreased.

Discussion

Meiofaunal composition and abundance

The microcosm meiofauna was similar in abundance to natural marine meiofauna but differed from it in composition. In marine subtidal sands, the meiofaunal biomass amounts to 1–2 g dry mass·m^{-2} and nematodes regularly dominate the meiofauna, comprising 50% of the total meiofaunal abundance, whereas copepods comprise only 7–12% (Coull 1988).

The meiofauna in both the microcosms and the sand filters were composed mainly of prehensile epibenthic species, such as the harpacticoid *Pseudonychocamptus*, and strongly adhesive species, such as the nematode *Anticoma*. *Pseudonychocamptus proximus* accounted for 79% of all specimens and 88% of the meiofaunal mass. There were only a few mesopsammic species such as the harpacticoid *Paramphisciella*. Sand in the sand filters was strongly backwashed every 5 days or so and may have prevented the colonization by mesopsammic species. In addition, water was forced through the sand in both the microcosms and the sand filters, possibly favoring epibenthic species at the expense of mesopsammic ones, hence the observed scarcity of nematodes, which may explain the results obtained in our experiments. Nematodes were shown to be largely responsible for increasing the mineralization rate of organic matter (Tenore et al. 1977; Alkemade et al. 1992).

Most meiofaunal species found here eat diatoms, microalgae, or bacteria. *Anticoma*, for example, is a selective deposit feeder (Wieser 1959). *Pseudonychocamptus* feeds not only on microalgae and diatoms, but also on bacterial exopolymer (Decho and Moriarty 1990). *Tisbe furcata* prefers bacteria (Vanden Berghe and Bergmans 1981). Our observa-

© 1999 NRC Canada

Fig. 4. Linear regression models of apparent mineralization rate (AMR) and meiofaunal biomass for the tryptone, detritus, and fishmeal experiments. Open circle in tryptone represents the microcosm excluded from analyses on the basis on unusually high mortality rate.

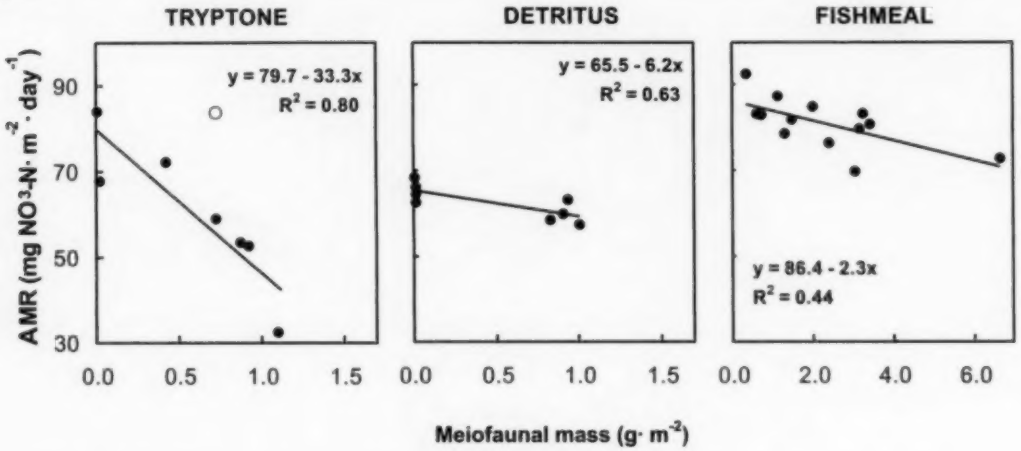


Table 4. Linear regression models of the mineralization experiments.

Experiment	Model	n	F	p	R^2	95% confidence interval of the slope
Tryptone	AMR = 79.6–33.3 Biomass	7	19.5	0.01	0.80	$-52.7 < \beta_1 < -13.9$
Detritus	AMR = 65.5–6.2 Biomass	8	10.4	0.02	0.63	$-10.9 < \beta_1 < -1.48$
Fishmeal	AMR = 86.4–2.3 Biomass	13	8.5	0.01	0.44	$-4.06 < \beta_1 < -0.57$

Note: AMR, expressed in mg NO_3-N·m^{-2}·day^{-1}; meiofaunal biomasses, expressed in g dry mass·m^{-2}.

tions indicate that hydrozoans live in the pools and are only transients in the sand filters.

Conditions of the experiments

A significant amount of the organic nitrogen was mineralized in all three experiments: 29% of the fishmeal, 32% of the detritus, and 80% of the tryptone. The measured AMR was probably not dependent on the amount of mineralizable substrate because the production rate of nitrate was relatively constant in the experiments. Organic nitrogen mineralization usually follows first-order kinetics (Stanford and Smith 1972) and the typical curve for net nitrogen mineralization has a parabolic shape (Campbell et al. 1993). We did not observe this phenomenon here, mostly because the experiment was not long enough for the AMR to become dependent on the available substrate.

The observed variability of AMR was not correlated to protozoan density. Protozoans are known to affect mineralization (Pomeroy 1970; Barsdate et al. 1974), but either they equally affected all microcosms or their effect was too small compared with that of meiofauna.

Linear regression models

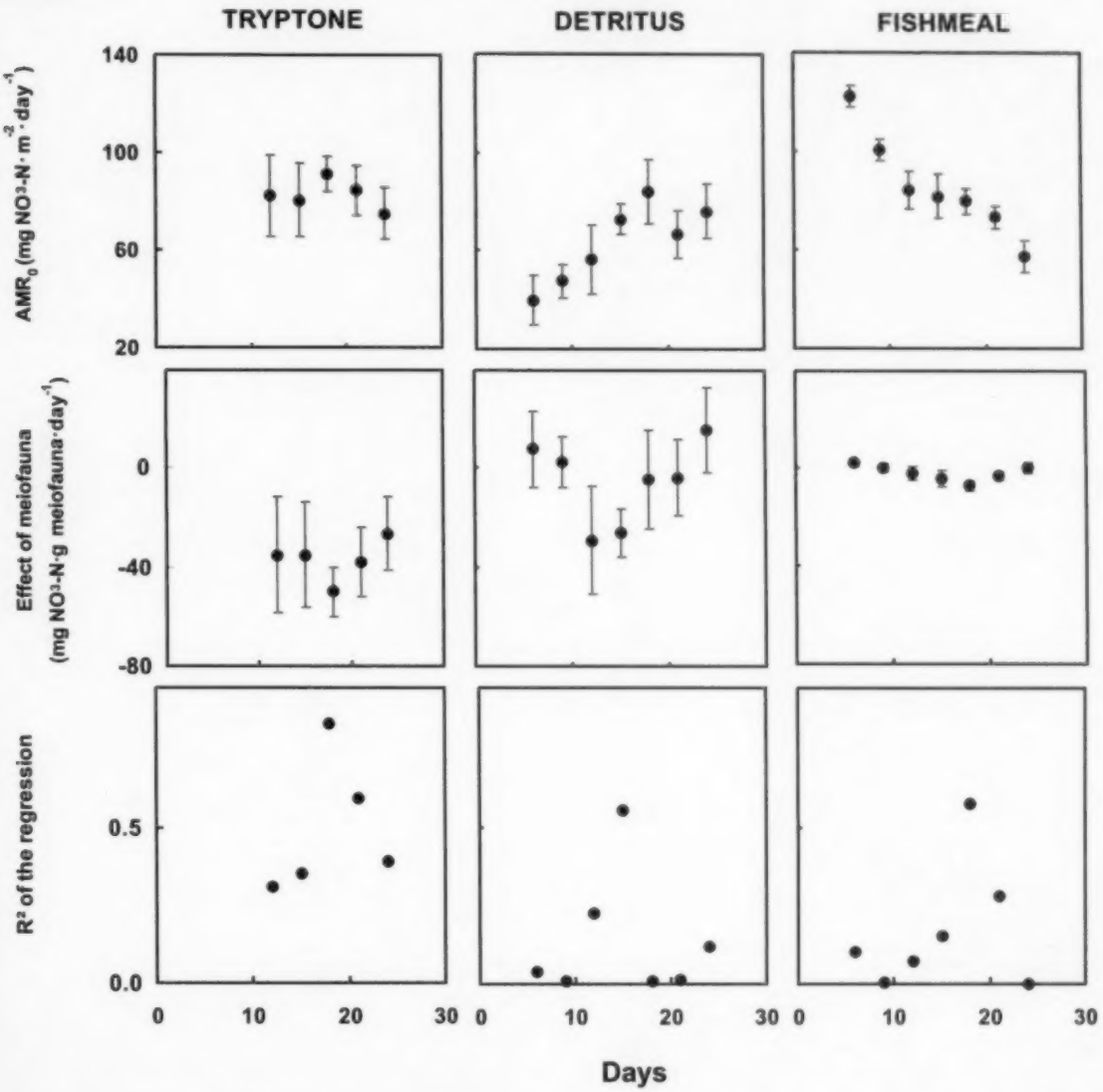
Linear regressions were used to describe the effect of meiofauna on AMR because we postulated that grazing was the main biological mechanism involved in our experiments and grazing rate per capita was probably constant over a wide range of meiofaunal masses. Other processes, e.g., bactericulture and bioturbation, were not considered important because water was forced through the sand. Fragmentation of food by meiofauna was considered negligible because of the detritus small particle size.

All three models show that meiofauna in the sand filters decreased the AMR in our microcosms where C:N ratios ranged from 4.4 to 8 (Fig. 4). These results confirm the idea that meiofauna feed little on detritus and extensively on bacteria (Meyer-Reil and Faubel 1980; Montagna 1995). Meiofauna also feed on diatoms, but none was present in the heterotrophic microcosms. There are therefore situations where marine meiofauna does not increase the mineralization rate of organic matter. This probably also occurs in streams because common stream-dwelling copepods reduce the density of detritally associated bacteria by as much as 58% (Perlmutter and Meyer 1991).

The AMR_0 for detritus (66 mg NO_3-N·m^{-2}·day^{-1}) is significantly lower than the AMR_0 for fishmeal (86 mg NO_3-N·m^{-2}·day^{-1}), even though more organic matter was present at the start of the detritus experiment. We think there are three reasons for this. First, detritus was more variable and slightly larger in size than fishmeal. Second, at the start of the experiment, the C:N ratio of detritus was higher than the C:N ratio of fishmeal (Table 1). At C:N ratio less than 25, mineralization increases exponentially as C:N decreases (Parnas 1975). DeAngelis (1992) estimated that mineralization increases threefold when the C:N ratio decreases from 8 to 4. Third, fishmeal is more labile than detritus. Fresh fishmeal was used in the microcosms, whereas detritus was comprised of unused food, unabsorbed food, and refractory

© 1999 NRC Canada

Fig. 5. Detailed analysis of the experiments showing the AMR_0 (mean ± SE), the effect of meiofauna (mean ± SE), and the R^2 on days 6, 9, 12, ..., and 24 for each experiment. AMR_t of each microcosm, i.e., AMR on days t = 6, 9, 12, ..., 24, were calculated by regressing five nitrate concentrations (t–6, t–3, t, t+3, and t+6) over time. Each set of AMR_t was then regressed against meiofaunal biomass.



objects such as scales and feathers that were left in the microcosms for almost 3 months before the start of the experiment.

Results of the covariance analysis suggest that the effect of meiofauna is independent of the C:N ratio of organic matter but dependent of the particle size (Table 1, Fig. 4). Meiofauna had the same effect on AMR in the fishmeal and detritus experiments, which shared similar particle sizes but different C:N ratios. Meiofauna had a significantly different effect on AMR in the fishmeal and tryptone experiments, which shared similar C:N ratios but different particle sizes.

The covariance analysis showed that the C:N ratio of organic matter significantly affected the AMR_0 (Table 1, Fig. 4). Bacteria mineralize organic matter with a low C:N ratio faster than that with a high C:N ratio and labile organic matter more easily than refractory organic matter (Parnas 1975).

Harpacticoid copepods, especially *Pseudonychocamptus proximus* that accounts for 98% of the meiofaunal biomass in our system, are mainly responsible for the observed effect of meiofauna on AMR. They comprise up to 98% of the meiofaunal biomass in our system. Other taxa such as rotifers are known to graze on bacteria (Thane-Fenchel 1968), but in this case, we suspect that they played a minor role or

© 1999 NRC Canada

no role at all because of their scarcity. Indeed, AMR was not related to the residual meiofaunal biomass (meiofaunal biomass – harpacticoid biomass) in the tryptone experiment ($R^2 = 0.026$, $F = 0.13$, $p = 0.73$).

The linear regression models show that the effect of meiofauna varied with the substrate (Table 4). Meiofauna (1 g dry mass·m^{-2}) reduced the AMR_0 of the fishmeal by 2.7%, the AMR_0 of detritus by 9.4%, and the AMR_0 of tryptone by as much as 42%. The AMR_0 of the experiments, which reflect bacterial activity in the absence of meiofauna, explain the observed variability in the effect of meiofauna. The AMR_0 of fishmeal (86 mg NO_3-N·m^{-2}·day^{-1}) was significantly higher than the AMR_0 of detritus (65 NO_3-N·m^{-2}·day^{-1}). Mineralizing bacteria, being more productive in the fishmeal experiment, better resist the grazing effect of meiofauna. The same explanation can be given in part for the tryptone experiment. The AMR_0 of tryptone (80 NO_3-N·m^{-2}·day^{-1}) was lower than that of fishmeal but higher than that of detritus. We suspect that the grazing rate of meiofauna was higher in the tryptone experiment because meiofauna could feed on nothing else but bacteria. Harpacticoids are capable of direct assimilation of small organic compounds such as glucose but probably not of complex dissolved organic matter such as tryptone (Hicks and Coull 1983).

Phases of the experiments

Through grazing, copepod-dominated meiofauna better prepared bacterial colonies for an influx of organic matter but then took a heavier toll on their production (Fig. 5). Meiofauna had a small positive effect on the AMR during the early phase of the detritus and fishmeal experiments when the reaction was starting: the substrate was plentiful with a low C:N ratio but mineralizing bacteria were not yet taking full advantage of it. Afterwards, during the middle phase, the negative effect of meiofauna was strongest when AMR_0 was at its highest and the C:N ratio was still low. Finally, as the C:N ratio increased, the AMR_0 decreased and meiofauna had a diminishing effect on AMR_0. The fact that meiofauna had less effect on mineralization during the late phase is probably an artifact produced by the differences in mineralization rates in the microcosms. The more meiofauna there was in a microcosm, the less substrate was mineralized during the middle phase and the more substrate with a lesser C:N ratio was available in the late phase. The result was that more mineralization took place during the late phase in microcosms having more meiofauna, producing an apparent reduced or, in the case of detritus, an apparent positive effect by the meiofauna.

Meiofauna exerted its negative effect even when very little substrate had been mineralized (Fig. 5). The negative effect of meiofauna appeared on day 12 in the detritus experiment, when only 12% of the organic nitrogen had been mineralized, and in the fishmeal experiment on day 9, when only 10% of the organic nitrogen had been mineralized.

Changes in the impact of meiofauna on mineralization during the three phases are easy to understand for the tryptone and fishmeal experiments because these substrates were added just prior to the experiment. However, because detritus were added 87 days before the detritus experiment, we would have expected a constant AMR_0 and a constant meiofaunal effect throughout the experiment. We suggest that changing 70% of the water before each experiment was very important in stimulating mineralization in the microcosms. The microcosms were in a closed circuit and no physicochemical filtration, such as granular activated carbon or ozone, was used. Seawater was yellow before it was changed and colorless afterwards. Refractory by-products of mineralization, such as phenols and humus, are known to build up in aquaria and to impart the yellow coloration to old aquarium seawater (Spotte 1991). They were also possibly reducing the AMR before each experiment.

The results of the early phase support the suggestion that meiofaunal grazing is in equilibrium with microbial production (Montagna 1984, 1995). The observed null or slightly positive effect of meiofauna means that bacterial production increased in microcosms with large meiofaunal biomass and adjusted to a larger grazing pressure. However, the results of the middle phase show that the influx of organic matter ruptured the equilibrium between microbial production and meiofaunal grazing. Bacterial productivity decreased in microcosms with large meiofaunal biomass and this resulted in a net negative effect of meiofauna on AMR (Fig. 5). The results of the late phase suggest a return to equilibrium as the added organic substrate became depleted. It therefore seems that the equilibrium between meiofaunal grazing and bacterial production is produced by a steady influx of organic matter rather than by a variable grazing rate per meiofaunal capita.

The strongest short-term effect of meiofauna was –50 mg NO_3-N·m^{-2}·day^{-1} per gram meiofaunal dry mass·m^{-2} for tryptone, –29 for detritus, and –7.3 for fishmeal (Fig. 5). These effects were observed when only 12% of the detritus and 10% of the fishmeal had been mineralized. Our results therefore suggest that when the substrate is plentiful and the water is relatively clean, meiofauna actually has a very strong impact on AMR. The strongest significant effect of 1 g meiofaunal dry mass·m^{-2}, as measured in the middle phase, was 9% reduction in AMR for fishmeal ($R^2 = 0.58$), 36% reduction for detritus ($R^2 = 0.56$), and 55% for tryptone ($R^2 = 0.83$). This suggests that less than 3 g meiofaunal dry mass·m^{-2} would have completely stopped the mineralization in the microcosms.

Microcosms compared with the sand filters

The regression model of the detritus experiment showed the effect of 1 g meiofaunal dry mass·m^{-2} on the mineralization of detritus to be 9.4% (Table 4). Meiofaunal biomasses of 20 to 40 g dry mass·m^{-2} have been found in the top 2.5 cm of sand in the sand filters of the St. Lawrence mesocosm in 1996. If our results in the microcosms were extrapolated to the sand filters, we would conclude that little or no mineralization of organic matter occurred there.

However, it is impossible to simply extrapolate our results to the sand filters because the conditions are different. The AMR_0 is probably higher in the sand filters than it was in the microcosms. Water in the mesocosm is ozone treated, there is a constant influx of fresh organic matter in the sand filters, and the filters are frequently backwashed. As a result, the organic matter is kept at its maximum lability, which permits the highest AMR_0. Moreover, there is a constant influx of algae in the sand filters, an ingredient absent from the detritus experiment. Harpacticoid copepods, especially

© 1999 NRC Canada

Pseudonychocamptus, are known to prefer feeding on live or recently dead algae and therefore exert less pressure on mineralizing bacteria (Rudnick 1989; Decho and Moriarty 1990).

Consequently, we cannot conclude that meiofauna exerts a strong negative impact on AMR in the St. Lawrence mesocosm. To examine this question, in situ experiments would be required, especially in the deeper parts of the filter sand where meiofauna is abundant and less algae and more refractory organic matter are probably present.

One reason why meiofauna did not have a positive effect in the microcosms is that all beneficial action from meiofauna was eliminated in the microcosms and in the sand filters: there are few nematodes; water flow through the sand leaves no role for bioturbation, which is thought to be the main mechanism by which meiofauna exerts its positive action (Alkemade et al. 1992); and there can be little bactericulture because the sand is frequently backwashed. All that is left is possibly fragmentation and a fecal pellet effect by the copepods. These effects might be negligible compared with the grazing action of meiofauna.

Meiofauna, therefore, does not always increase the mineralization rate of organic matter. Copepod-dominated meiofauna decrease nutrient regeneration rates in heterotrophic habitats when the C:N ratios are low. The effect of meiofauna on the mineralization rate of organic matter can be seen as a delicate balance between negative and positive processes, such as grazing, bioturbation, bactericulture, and fragmentation. The net result of these processes, an increase or a decrease in the mineralization rate of organic matter, depends on environmental factors, such as filtration and heterotrophy in closed systems, and on faunal composition, two sets of factors that cannot easily be separated one from each other.

Acknowledgments

This research was supported by the Montreal Biodome and a grant to A. Morin from the Natural Sciences and Engineering Research Council of Canada. We gratefully acknowledge the major contributions of Dr. Judith Fournier (Polychaetes), Dr. D. Calder (Hydrozoans), Dr. Ilse Bartsch (Acarians), and M. Clément (Harpacticoids) for their taxonomic determinations. We would also like to thank R. Léger, Head of Live Collections at the Montreal Biodome, S. Pépin, Curator of aquatic animal collections, and the aquarium and engineering staff of the Montreal Biodome for their help and cooperation. Brian Colwill, C.Tr., kindly revised the English style and grammar and Dr. D. Gagnon and two anonymous referees provided helpful comments on an earlier version of this paper.

References

Alkemade, R., Wielemaker, A., de Jong, S.A., and Sandee, A.J.J. 1992. Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina angli*. Mar. Ecol. Prog. Ser. **90**: 149–155.

Aller, R.C., and Aller, Y.C. 1992. Meiofauna and solute transport in marine muds. Limnol. Oceanogr. **37**: 1018–1033.

APHA, AWWA, and WEF. 1992. Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association, Washington, D.C. pp. 4–91.

Barsdate, R.J., Prentki, R.T., and Fenchel, T. 1974. Phosphorus cycle of model ecosystems: significance for decomposer food chains and effect of bacteria grazers. Oikos, **25**: 239–251.

Campbell, C.A., Ellert, B.H., and Jame, Y.W. 1993. Nitrogen mineralization potential in soils. *In* Soil sampling and methods of analysis. Chapt. 33. *Edited by* M.R. Carter. Lewis Publishers, London. pp. 341–349.

Coull, B.C. 1988. Ecology of the marine meiofauna. *In* Introduction to the study of meiofauna. Chapt. 3. *Edited by* R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 18–38.

DeAngelis, D.L. 1992. Dynamics of nutrient cycling and food webs. Chapman & Hall, London.

Decho, A.W., and Moriarty, D.J.W. 1990. Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. Limnol. Oceanogr. **35**: 1039–1049.

Difco 1984. Difco manual. Dehydrated culture media and reagents for microbiology. 10th ed. Difco Laboratories, Detroit, Mich. p. 1027.

Epstein, S.S., and Shiaris, M.P. 1992. Rates of microbenthic and meiobenthic bactivory in a temperate muddy tidal flat community. Appl. Environ. Microbiol. **58**: 2426–2431.

Feller, R.J., and Warwick, R.M. 1988. Energetics. *In* Introduction to the study of meiofauna. Chapt. 13. *Edited by* R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 181–196.

Fenchel, T. 1978. The ecology of micro- and meiobenthos. Annu. Rev. Ecol. Syst. **9**: 99–121.

Findlay, S., and Tenore, K.R. 1982. Effect of a free-living marine nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. Mar. Ecol. Prog. Ser. **8**: 161–166.

Findlay, S.E.G. 1981. Small scale spatial distribution of meiofauna on a mud- and sandflat. Estuarine Coastal Shelf Sci. **7**: 255–268.

Heip, C., Vincx, M., and Vranken, G. 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol. Annu. Rev. **23**: 399–489.

Hicks, G.R.F., and Coull, B.C. 1983. The ecology of marine meiobenthic harpacticoid copepods. Oceanogr. Mar. Biol. Annu. Rev. **21**: 67–175.

Lee, N.M., and Welander, T. 1994. Influence of predators on nitrification in aerobic biofilm processes. Water Sci. Technol. **29**: 355–363.

Meyer-Reil, L.-A., and Faubel, A. 1980. Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. Mar. Ecol. Prog. Ser. **3**: 251–256.

Montagna, P.A. 1984. *In situ* measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. Mar. Ecol. Prog. Ser. **18**: 119–130.

Montagna, P.A. 1995. Rates of metazoan meiofaunal microbivory: a review. Vie Milieu, **45**: 1–9.

Parker, D., Lutz, M., Dahl, R., and Bernkopf, S. 1989. Enhancing reaction rates in nitrifying trickling filters through biofilm control. J. Water Pollut. Control Fed. **61**: 618–631.

Parnas, H. 1975. Model for decomposition of organic material by microorganisms. Biol. Biochem. **7**: 161–169.

Perlmutter, D.G., and Meyer, J.L. 1991. The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. Ecology, **72**: 2170–2180.

Pfannkuche, O., and Thiel, H. 1988. Sample processing. *In* Introduction to the study of meiofauna. Chapt. 9. *Edited by* R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 134–145.

Pomeroy, L.R. 1970. The strategy of mineral cycling. Annu. Rev. Ecol. Syst. **1**: 171–190.

© 1999 NRC Canada

Rudnick, D.T. 1989. Time lags between the deposition and meiobenthic assimilation of phytodetritus. Mar. Ecol. Prog. Ser. **50**: 231–240.

Stanford, G., and Smith, S.J. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. Proc. **36**: 465–472.

Spotte, S. 1991. Captive seawater fishes. Science and Technology. John Wiley & Sons, Toronto, Canada.

Tenore, K.R., Tietjen, J.H., and Lee, J.J. 1977. Effect of meiofauna on incorporation of aged Eelgrass, *Zostera mari*na, detritus by the polychaete *Nephthys incisa*. J. Fish. Res. Board Can. **34**: 563–567.

Thane-Fenchel, A. 1968. Distribution and ecology of non-planktonic brackish-water rotifers from scandinavian waters. Ophelia, **5**: 273–297.

Vanden Berghe, W., and Bergmans, M. 1981. Differential food preferences in three co-occurring species of *Tisbe* (Copepoda, harpacticoida). Mar. Ecol. Prog. Ser. **4**: 213–219.

Wieser, W. 1959. Free-living marine nematodes. IV. General part. Lunds Universitets Arsskrift, Ny Foljd Audelning 2, **55**: 1–111.

Yingst, J.Y., and Rhoads, D.C. 1980. The role of bioturbation in the enhancement of bacterial growth in marine sediments. *In* Marine benthic dynamics. *Edited by* K.R. Tenore and B.C. Coull. University of South Carolina Press, Columbia, S.C. pp. 407–421. (Belle W. Baruch Libr. Mar. Sci., v. 11.)

© 1999 NRC Canada

PERSPECTIVES

Fisheries assessment: what can be learned from interviewing resource users?

Barbara Neis, David C. Schneider, Lawrence Felt, Richard L. Haedrich, Johanne Fischer, and Jeffrey A. Hutchings

Abstract: Fishers have detailed knowledge of their resources, their environment, and their fishing practices that is rarely systematically collected. We conducted three types of interviews with coastal Newfoundland fishers to identify the range of information available, to see if it could be quantified, and to explore its potential for reconstructing trends within fisheries. These fishers have many terms for Atlantic cod (*Gadus morhua*), each associated with characteristic patterns of seasonal movement and availability to gear and indicating the location of several coastal spawning areas. They described a variety of changes in fishing practice. Of the four changes that could be quantified, all contributed to decadal-scale increases in catch efficiency prior to 1992, while change in catch per unit of effort for cod was consistently negative at decadal scales. For these fishers' lumpfish (*Cyclopterus lumpus*) roe fishery, catch per unit of effort was consistently negative in the 1990s. We describe ways to access the large reservoir of information held by fishers, the use of several cross-checks to identify consistent patterns, and the use of trends and patterns to broaden the basis for interpreting quantitative surveys used in fisheries assessment. Local information from resource users can be assembled in forms usable in quantitative stock assessments.

Résumé : Les pêcheurs possèdent au sujet de leurs ressources, de leur milieu et de leurs pratiques de pêche des connaissances détaillées qui sont rarement recueillies de façon systématique. Nous avons mené auprès de pêcheurs côtiers de Terre-Neuve trois types d'entrevues pour déterminer l'étendue de l'information disponible, voir s'il était possible de la quantifier et explorer son potentiel pour tenter de reconstituer les tendances présentes dans les pêches. Ces pêcheurs possèdent plusieurs termes pour décrire la morue *Gadus morhua*, dont chacun est associé à des caractéristiques particulières des déplacements saisonniers et de la vulnérabilité aux engins de pêche, et ils ont indiqué l'emplacement de plusieurs frayères côtières. Ils ont décrit un grand nombre de changements dans les pratiques de pêche. Les quatre changements qui ont pu être quantifiés ont tous contribué à des augmentations d'échelle décennale dans l'efficacité de capture avant 1992, tandis que le changement dans les captures par unité d'effort (CPUE) pour la morue était régulièrement négatif à l'échelle décennale. Dans la pêche de la lompe *Cyclopterus lumpus* pour les oeufs, pratiquée par ces pêcheurs, le CPUE était régulièrement négatif dans les années 90. Nous décrivons des façons d'accéder au vaste réservoir d'information détenu par les pêcheurs, l'emploi de plusieurs recoupements pour repérer les patrons réguliers, et le recours aux tendances et aux patrons pour élargir la base d'interprétation des relevés quantitatifs employés dans les évaluations. L'information locale fournie par les utilisateurs de la ressource peut être rassemblée sous une forme utilisable pour les évaluations quantitatives des stocks.

[Traduit par la Rédaction]

Introduction

Resource users develop detailed knowledge of their resources, their environments, and their fishing practices. This information has long been used by some fisheries scientists as background knowledge (Smith 1994; Hutchings et al. 1997). It is regularly used to generate testable hypotheses (e.g., Taggart and Frank 1987). Resource users' knowledge is rarely collected in a systematic fashion. When it is, highly structured formats (telephone surveys, logbook programmes)

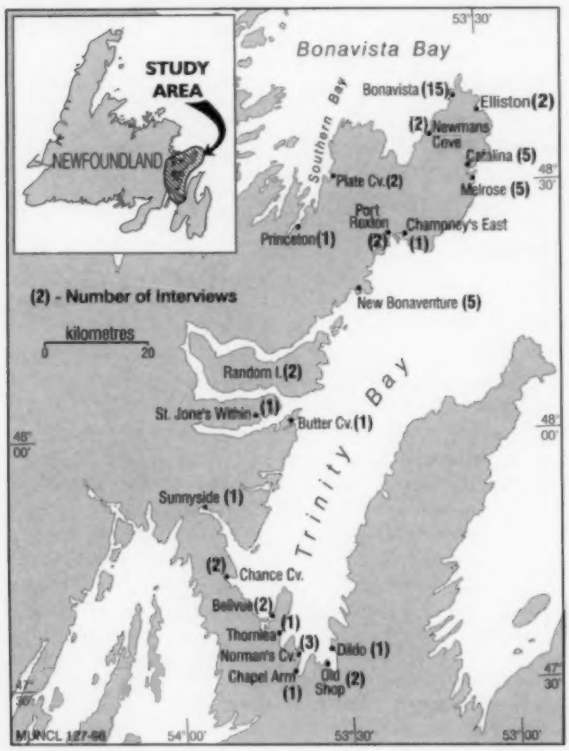
Received July 10, 1998. Accepted June 22, 1999.
J14678

B. Neis[1] **and L. Felt.** Department of Sociology, Memorial University of Newfoundland, St. John's, NF A1C 5S7, Canada.
D.C. Schneider. Department of Biology, Memorial University of Newfoundland, St. John's, NF A1B 3X7, Canada.
R.L. Haedrich and J. Fischer. Eco-Research Project, 202 Elizabeth Ave., Memorial University of Newfoundland, St. John's, NF A1B 5S7, Canada.
J.A. Hutchings. Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.

[1]Author to whom all correspondence should be addressed. e-mail: bneis@morgan.ucs.mun.ca

© 1999 NRC Canada

Fig. 1. Study area and geographic distribution of 56 interviews with inshore and longliner fishers.



tap only a limited range of the information present, and programmes often suffer from inconsistent participation. In general, little of fishers' information has been used, relative to what is present or to what might be used in resource management (Berkes 1993; Pinkerton 1994).

Personal interviews with fishers can elicit large amounts of information on both commercial and noncommercial species related to fish behaviour and fishing practices. Hutchings (1996) identified three ways in which information from guided personal interviews could be used in scientific assessments. First, local knowledge of the dates when fish are caught in fixed-gear locations can provide information on seasonal and directional fish movements. Second, fishers can provide information pertaining to stock structure. Fishers can provide information on movement patterns (through catch patterns), spawning grounds (presence of females in ripe and running condition), juvenile habitat (e.g., bycatch of juvenile Atlantic cod (*Gadus morhua*) in caplin traps), and spatial patterns in fish morphology. This information is useful in conjunction with genetic information (e.g., Ruzzante et al. 1996), tagging experiments (e.g., Lear 1984), and morphometric studies used in identifying stocks. Third, catch rate data obtained from fishers have the potential to reflect local changes in fish abundance (Hutchings and Myers 1994). This list of uses can be extended. For example, fishers can provide a full account of their activity and observations including the distribution of effort and changes in fishing practice that need to be examined when catch per unit of effort (CPUE) is used to track stock size. Fishers can contribute to quantitative surveys of abundance, as in acoustic surveys of herring stocks in the Bay of Fundy (Stephenson 1998). Fishers can identify former as well as current spawning areas (Ames 1998). Information from fishers can be used to identify changes in terminal mortality (mortality rate in the oldest age-class), a parameter that is poorly estimated in research surveys and that notoriously leads to overestimation of stock size in assessments based on virtual population analysis or related forms of population reconstruction (Hilborn and Walters 1992).

The body of information held by fishers has an important role to play in fisheries assessment. When this body of information matches scientific assessments, uncertainty is reduced and assessments become more convincing to resource users. When the two sources of information diverge, information from both sources needs to be reexamined (Evans 1996). Yet while this body of information has a role to play, there remain practical impediments to its use. Scientific terms do not match the terms that fishers use to organize their knowledge (Johannes 1993). The geographic range of information from each fisher is limited. Knowledge is unevenly distributed among fishers, being more concentrated among older fishers and skippers. It is largely oral, rather than written, and subject to the effects of memory loss.

We describe a systematic means of assembling and cross-checking information from fishers based on three kinds of interview: one to define terms, one that allows geographically limited information collected by individuals during their careers to be assembled to identify recurrent patterns of change, and one that allows verification, refinement, and updating of information on particular fisheries. To illustrate this approach, we report the results from personal interviews with fishers in the Bonavista and Trinity Bay, Newfoundland, area inshore small-vessel (<35 ft) and nearshore larger-vessel (>35 ft) fisheries (Fig. 1). Our objectives were to (*i*) demonstrate both the amount and types of information that result from personal interviews informed by knowledge of local terms for fish and fishing areas, (2) use this information to document the diversity of changes in fishing practices that had taken place in local fisheries during fishers' careers, (3) determine whether any of these changes in practice could be quantified, and (4) determine whether trends in CPUE can be identified by assembling results from detailed personal interviews.

Methods

This research is based on results from two types of personal interviews and followup telephone interviews conducted with inshore and nearshore fishers living on the northeast coast of Newfoundland. The personal interviews were conducted in the summers of 1994 and 1995, roughly 2 years after the announcement of the moratorium on northern cod, and followup telephone interviews were conducted in December 1995 and 1996. Effectively accessing fishers' knowledge requires shared understanding of local terms for fish and fishing grounds and for fishing gear (Johannes 1993); thus, we began with 10 taxonomy/toponomy interviews. The main body of interviews consisted of personal interviews with 56 local fishers. Followup telephone interviews with a subsample of these fishers were used to supplement data on the lumpfish (*Cyclopterus lumpus*) roe fishery collected in the first set

© 1999 NRC Canada

of interviews. Results from the research were presented at local feedback meetings attended by some study participants and some who had not participated in the study. Given the relatively small sample size and the limits of snowball sampling, we do not use inferential statsitics to generalize from these fishers to the larger population of fishers in the area or in Newfoundland as a whole.

The study area included the region between the communities of Princeton, Bonavista Bay, and Dildo, Trinity Bay (see Fig. 1). The area was chosen to capture a range of fishing locations from inner bays to outer headlands and a range of fishing technologies from coastal small boats to offshore vessels, as well as to relate findings to a wider study on the sustainability of coastal communities ongoing at Memorial University of Newfoundland.

Sampling design

Random sampling from a defined list of licensed fishers within the study area was not possible because of the absence of an accurate, up-to-date list of licensed fishers for the entire study area. It is also not recommended for this type of research. Many studies of resource users' ecological knowledge, including our own, employ snowball sampling to identify local "experts." Information is unevenly distributed among fishers, being concentrated among skippers, particularly those with long careers, those who are especially observant, and those who keep records (Mailhot 1993). Snowball sampling generates an ever-increasing set of interviews through a referral process in which interviewees are asked to provide additional, appropriate names for interviewing (Babbie 1989). For the taxonomy/toponomy interviews, we asked local fishers to recommend primarily older, retired fishers distributed throughout the study area. For the main body of personal interviews, we sampled throughout the study area with greater intensity in areas with larger numbers of fishers and concentrated on skippers. The sample was stratified by sector (inshore and nearshore) but weighted towards the inshore, <35 ft vessel sector, which has the largest number of boats in this area. Among those interviewed, fishing experience ranged from a maximum of 60 to a minimum of 12 years (six at 51–60 years, nine at 41–50 years, 13 at 31–40 years, 16 at 21–30 years, 22 at 12–20 years). Most (48) had served as skippers; another five were partners in enterprises without clearly designated skippers. The remainder had either served exclusively as crewmembers or their status in fishing vessels was not indicated in the transcripts. Followup telephone interviews were done with fishers who indicated in the original interviews that they had participated in the lumpfish roe fishery (n = 22).

Interview strategy and questions

Personal interviews were designed and carried out by a team of researchers involving social and natural scientists. At least two interviewers were involved, with one conducting the interview and the second helping the fisher record spatial information and monitoring the schedule to ensure that key areas were not missed. With the written permission of the fisher, personal interviews were taped and subsequently transcribed. They included a mapping component with spatial data recorded on nautical charts with Mylar overlays. Interviews lasted between 1.5 and 4 h and had a semistructured format in that they were guided by an interview schedule but fishers were given the opportunity to introduce information and to guide, somewhat, the course of the interview. Where fishers approved, tapes, transcripts, and digitized maps and Mylars are being archived in the Memorial University of Newfoundland Folklore Archives.

For the taxonomy/toponomy interviews, fishers were shown diagrams of fish and bird species and asked to identify those observed in their areas, provide local names for the species that they were familiar with, and locate, provide local toponyms for, and describe local spawning areas and fishing grounds.

The main body of 56 personal interviews had a career-history format and were guided by an interview schedule. The career-history interviews opened with basic demographics and information on training. Data were collected on all of the licenses held and vessels owned, when they were owned, as well as descriptions of engines, gear, and equipment used on each vessel. The timing of vessel ownership helped fishers reconstruct their fishing careers. Fishers were asked to describe a typical fishing season — species fished, timing, place, gear fished, landings — at the beginning of their careers, at one or two points of change during their careers, and at the end of their careers or just prior to the moratorium. Demographic information and information on fishing licenses, boats, and gear was summarized on a short questionnaire during these interviews. In most cases, the data recorded were from memory. In a few cases, fishers produced personal logbooks, which were then used as well. Career-history interviews provide much contextual information that increases the likelihood of accurate interpretation of responses, and they allow the interviewee to determine, to some degree, the direction of the interview, thereby accessing issues, observations, and interpretations that were not anticipated by the interviewer.

Observations are often correct, even when interpretations differ (Gunn et al. 1988). The main focus of the questioning was on what fishers were fishing for, what they were fishing with, where they were fishing, and what they observed. Interpretations of, for example, trends in fish abundance and causes of those trends were also sought, but these did not substitute for information on actual observations. For a more detailed discussion of fishers' knowledge and alternative methodologies for accessing it, see Neis et al. (1999).

Structured followup telephone interviews were done with a subsample of the original group of fishers who indicated that they had participated in the lumpfish roe fishery. These gathered more precise and more standardized information on catch and effort for this particular fishery and allowed us to update the information on an annual basis for 2 years.

We reported our results at followup meetings with fishers in the region. These meetings allowed us to check our findings with local fishers, including some who were not in the original sample, and provided some additional data. On-the-water discussions with 10 fishers from Melrose and Chance Cove, Trinity Bay, and a resulting coauthored report with these fishers provided additional data on fisheries in these areas and another check on some of the data in the original interviews (Fischer et al. 1997).

Data analysis

Stock distinctiveness is a central component of fisheries assessment. In a mixed-population fishery, small populations with high catchability risk progressive depletion (Hutchings et al. 1993). To assemble information on stock distinctiveness, we documented fishers' taxonomic terms for Atlantic cod and then used these to retrieve and interpret observations on the timing, location, and depth of particular cod fisheries, observations on the timing and direction of cod movements, and observations of cod spawning as indicated by cod with eggs or milt (fishers refer to this as "milk") "running" at the point of capture.

Changes in efficiency are a key piece of information in any fisheries assessment based on catch statistics, so it was of interest to attempt to quantify this change from the interview data. The standard way of quantifying efficiency is

$$C = qVB$$

where C is annual catch (tonnes per year), V is number of fishing units (vessels), and B is stock biomass (tonnes). Vessel efficiency q is called catchability in the literature; it has units of tonnes caught per tonne available, and for a vessel-year, q is percent per vessel-year. Efficiency q cannot be measured directly if catch C is being

© 1999 NRC Canada

used to estimate biomass B. The standard solution to this problem is to take the ratio of commercial catch C_{comm} to research vessel catch C_{rv} for the same stock B; hence the product of the catch and vessel ratios will equal the efficiency ratio:

$$(C_{comm}/C_{rv})(V_{comm}/V_{rv}) = q_{comm}/q_{rv}.$$

By holding research vessel efficiency q_{rv} constant from year to year, the product of the catch and vessel ratios becomes a measure of commercial efficiency. Both the catch and the vessel ratios are measured each year. Consequently, several years of data are required to detect changes in commercial efficiency by the standard method of calibrating commercial against research vessel catches. This lag will lead to overestimation of stock size if efficiency is rising (Pope 1977). The need for timely information on trends in effort (qV) has long been known. We examine whether more timely assessments of changing efficiency could be obtained by interviewing fishers directly rather than relying on calibration of commercial and research vessel catches across multiple years.

Information on changes in fishing efficiency was collected from the short questionnaire summary of career-history interviews and checked against the transcripts. This information was summarized in a database. We divided fishers into two sectors: inshore (vessels <35 ft) and nearshore (vessels >35 ft). Three fishers who switched sectors (a third fishing strategy) were removed from the analysis.

Inshore cod fishers were divided into three generations on the basis of their period of entry into the fishery (1920s–1930s, 1940s–1960s, 1970s–1980s) and their careers were divided into three periods: start, middle, and end. This approach allowed us to present both longitudinal and cross-sectional data and to control for a probable tendency for efficiency to increase within generations as a consequence of career-related increases in purchasing power. Average horsepower (n = 38) and vessel capacity (n = 37) were calculated for each generation and each period, along with average number of cod nets (gill nets used for cod) (n = 37) and average number of traps per crew (n = 37). Changes in the total number of gill nets and traps owned by fishers in this sample (1980–1992) were also plotted.

Career length differed among the three generations because of the announcement of the northern cod moratorium in 1992. We quantified change in efficiency, as an annual percentage, for two intervals: start of career (year) to midcareer (year) and midcareer to late career (year of retirement or moratorium). Where data permitted, percent change per year was calculated for vessel capacity (pounds) (n = 26), engine size (horsepower) (n = 29), boat length (feet) (n = 36), cod nets (maximum number of nets at one time) (n = 23), and traps (number owned) (n = 34). For boat length (BL) the annual change was calculated as

$$\Delta BL/year = (BL_{mid} - BL_{start})(BL_{start})^{-1} \times (Year_{mid} - Year_{start})^{-1}$$

and

$$\Delta BL/year = (BL_{end} - BL_{mid})(BL_{mid})^{-1} \times (Year_{end} - Year_{mid})^{-1}.$$

Both annual rates were then plotted against $Year_{mid}$. Similar calculations and plots were made for capacity, engine size, cod nets, and traps.

Vessel size for longliner (nearshore) fishers ranged from 38 to 64 ft and the main gear was gill nets. Where data availability permitted (seven out of nine fishers), we computed percent change in boat size, horsepower, capacity, gear amounts, and CPUE using the same formula as for inshore data.

Information on landings and changes in CPUE is also important in fisheries assessment. We analysed these data for both cod and lumpfish roe fisheries. For cod, fishers were asked to describe a good, average, and poor season of landings at different phases in their careers and, where possible, to provide remembered real landings for years just prior to the moratorium (n = 35).

We tabulated catches and effort (nets) and then computed change in nets (ΔNets), changes in catch (ΔC), and change in CPUE (ΔCPUE):

$$\Delta Nets = (Net_{final} - Net_{initial})/Net_{initial}$$

$$\Delta C = (C_{final} - C_{initial})/C_{initial}$$

$$\Delta CPUE = ((C_{final}/Net_{final}) - (C_{initial}/Net_{initial}))/(C_{initial}/Net_{initial}) = (1 + \Delta C)/(1 + \Delta Net) - 1.$$

Similar calculations were then made for the lumpfish roe fishery using data from career-history interviews with 22 fishers active in this fishery, supplemented by information from followup telephone interviews. We used the original interviews to examine whether the spatial scale of this fishery increased between 1980 and 1996. Change in CPUE was calculated as a percentage (ΔCPUE) and then taken as an annual rate by dividing by the number of years from the initial to final date. Per annum values were plotted against start date to examine changes in CPUE over time.

Results

Cod stock distinctiveness

The interview transcripts provided several kinds of information relevant to understanding stock structure: information on seasonal locations of cod, the direction and timing of movement, and spawning. Cod are locally referred to as "fish." These fishers distinguished between "capelin fish" and "herring fish." In the Bonavista area, the term "capelin fish" or "fish that come with the capelin" refers to the cod that arrive on the fishing grounds in late May – early June and are associated with the arrival of migrating capelin. These are generally described as "blackbacked" or "sunburnt" and are intercepted by the coastal trap and gillnet fisheries. Most are believed to migrate to the coast from "offshore," but this may include areas of Trinity and Bonavista bays outside the traditional inshore fishing grounds. Consistent with tagging data and based on information derived from tags that they had returned to the Department of Fisheries and Oceans (DFO), these fishers knew that these capelin fish could come from several offshore banks, from deeper areas closer to the Bonavista area, and sometimes from outside the bay.

Tagging research, as well as some landings data, and information on spawning locations suggest that some cod overwinter inshore in the study area (Hutchings et al. 1993; Taggart et al. 1995; Lilly 1996). Information supporting this conclusion came primarily from observations by fishers within Trinity and Bonavista bays, in contrast with those from the Bonavista area. The former refer to "herring fish," which follow herring into the arms and reaches of Bonavista and Trinity bays in the fall, after October, and then overwinter in these areas and disperse in pursuit of migrating herring in the spring (March–May). Herring cod were har-

© 1999 NRC Canada

Fig. 2. Identified locations for winter gillnetting of cod and observed cod spawning aggregations.

vested in deeper (16–24 fathoms, 1 fathom = 1.8288 m) cod traps and in gill nets in the spring and in gill nets when they returned to the arms with the herring in the fall. In the Chance Cove area, a late fall and early winter cod gillnet fishery took place at depths of between 80 and 150 fathoms in the late 1970s and early 1980s. In Princeton (Bonavista Bay), cod would begin moving back into Southern Bay in December and could be jigged and gillnetted through the ice up as far as Charleston until mid-January. Small winter fisheries involving jigging and gillnetting through the ice were reported in Bull Arm, Southwest Arm above Hatchet Cove, and in the Charleston area (Southern Bay), Bonavista Bay (see Fig. 2).

Another indication of overwintering fish was the appearance of smallish trap cod, brownish in colour, that were harvested in certain areas as early as mid-April and May, prior to the arrival of the capelin and the capelin fish. Believed to overwinter in Trinity Bay, these cod were smaller and occupied a different depth (in and around the rocks and kelp) than herring cod. They were harvested in shoal-water traps (8–12 fathoms). Larger, brownish cod have been observed in some abundance in shoal water since the moratorium.

Another term for cod used by fishers, "breeders" or "mother fish," referred to very large, pale or "white" fish, sometimes with large amounts of spawn in them. It was widely believed that these mother fish were fished out after the introduction of gill nets in the 1960s. Several fishers said the pattern of feeding and movement of the large, old breeders or mother fish seemed to differ from that of smaller fish. Fishers thought that these fish settled out and stayed in deep gullies or channels year-round rather than migrating offshore or inshore with the herring and caplin. Mother fish were found in the arms and deeper areas or "trenches" of Trinity and Bonavista bays and in the deeper water off the Bonavista headland in the 1950s and 1960s.

Previous research has documented cod spawning aggregations and individual spawning fish in various locations in Trinity and Bonavista bays (Hutchings et al. 1993; Potter 1996; Rose 1996; Smedbol and Wroblewski 1997). Fisher interviews described cod spawning in these general locations, but some additional locations were also identified. Mature cod are referred to by fishers as "spawny" or as "running" with eggs and milt. Figure 2 shows the locations of such cod identified in these interviews. These include an aggregation observed in a June–July gillnet fishery off Hopeall Head in 50–60 fathoms of water that was believed to have been fished out by the mid-1980s. A Chapel Arm fisher reported observing spawning cod on one occasion in a fall trap fishery in the early 1990s. Spawning cod were observed in a summer gillnet fishery northeast of the Bellevue Peninsula that targeted large breeders in deepwater in the 1960s (also see Hutchings et al. 1993). They were also observed in the June trap fishery near Thornlea. A Chance Cove fisher described gillnetting cod in 110–120 fathoms of water in the fall and winter in the late 1970s that included some big, spawny ones. He reported seeing the spawn running out on the ice. A spawning aggregation was observed on Heart's Ease Ledge in the summer months. Smedbol and Wroblewski (1997) found this aggregation by talking to fishers (K. Smedbol, Department of Biology, Memorial University of Newfoundland, personal communication) and then reported quantitative information. Fishers in the Butter Cove and St. Jone's Within areas also described catching spawning cod in the summer months in May or June. Most of the spawny cod that they would get would be in the gill nets.

In 1996, a spawning aggregation was "discovered" in Smith Sound (Fig. 1) during a scientific survey and then quantified acoustically (Rose 1996). Fishers described two spawning aggregations in the Smith Sound area: one observed in the 1960s, in association with the introduction of gill nets into an area above Bluff Point in Smith Sound in 30–50 fathoms of water, and the second fished more recently in the area off Hickman Harbour, adjacent to the Thoroughfare, in depths of 90 fathoms and more. Fishers followed this latter aggregation as it migrated out of Smith Sound to this spawning area. Fish were observed to be spawning in early May and mid-June, dispersing after mid-June. An older, retired fisher from Trinity described fishing trawls in 150–180 fathoms of water in the summer in the middle of Trinity Bay. Some of the cod that they caught in this fishery would be spawny. Similarly, some of the large breeders that they caught in the spring off Catalina were observed to be ready to spawn. These cod were also mostly from the deep water. A Bonavista fisher described a possible spawning aggregation located in a gulch 20–21 mi off Cape Bonavista in June or July during the 1970s (see Hutchings et al. 1993 for scientific documentation of spawning in these areas). Fishers

© 1999 NRC Canada

also reported observing spawning cod in the King's Cove and Princeton areas in May and June. In a separate set of interviews with Bonavista Bay fishers, Potter (1996) identified a spawning area for cod in Southern Bay. Some fishers observed that in recent years, they were finding cod spawning in shoaler water and at smaller sizes than in the past.

Efficiency

During the interviews and subsequent feedback meetings, fishers described a variety of changes in fishing practice during their careers. The sequence of boats and the length, engine power, and capacity of each were usually remembered, without recourse to written records. Many reported increases in boat length during their careers, but others stayed under the 35-ft limit for licensing reasons. More notable were changes in engine power, which was not regulated, and hence did not tend to stop at a fixed upper limit. Increases in engine power were accompanied by increases in boat capacity, longer trips from shore, and the introduction of mechanical hauling devices.

During the 1980s, electronic devices (navigational aides and sonar) became widespread. The effect that these have on fishing practice is profound, although not obvious. With exact positioning, a large number of gill nets can be left in the water between visits, greatly increasing the number of nets that can be fished per boat. Acoustic fish finders further increased the mobility of the gillnet fishery, allowing nets to be moved to areas known to have fish.

Changes in gear design and material also tended to increase efficiency. More durable material allowed gill nets to soak longer and reduced loss from torn traps. Most trap fishers changed the design of their traps from traditional to modified (winged), Japanese, or long-range traps (a rectangular Labrador design that could be set in previously unutilized berths) in the 1970s and 1980s. These changes, often associated with reductions in mesh size outside of the drying twine, are believed to have increased their catches by retaining smaller fish, increasing the number of possible berths, and allowing them to fish more traps efficiently by holding fish better. The introduction of sounders made it easier to fish a larger number of traps. Sounders reduce the time spent hauling traps by allowing small or zero catches to be identified before hauling. The introduction of power blocks reduced the time to empty a trap and increased the number of traps that a crew could haul in a day. Power blocks also made it easier to move large gear (traps) during the season, if catches at the first mooring were low, and allowed traps to be set in deeper water and where tidal currents require heavy moorings.

Of the many changes in practice, four were readily quantified: capacity (tonnes per vessel), gear (nets per vessel), engine power (horsepower = 2700 kJ/vessel-hour), and triptime (vessel-hours per vessel-year). The relationship of these components to overall efficiency q_{comm} is

$$q_{comm} = \text{gear}^a \times \text{capacity}^b \times \text{power}^c \times \text{triptime}^d \times q_{other}.$$

We have chosen to call q_{comm} efficiency rather than catchability because the term catchability implies changes in fish behaviour rather than changes in fishing practice and fish abundance.

Based on the principle of homogeneity of units, $b = -a$, $c = d$, and hence:

$$q_{comm} = \text{gear}^1 \times \text{capacity}^{-1} \times \text{power}^1 \times \text{triptime}^1 \times q_{other}$$

where q_{other} is tonnes per kilojoule per net-vessel, a measure of maximum delivery rate (tonnes per kilojoule) relative to nets fished by each vessel.

Figure 3 shows both generational and intergenerational trends in efficiency. Engine power increased throughout fishing careers, regardless of the generation of entry into the fishery (Fig. 3*a*). Capacity also increased, with greater change for third-generation fishers than for earlier generations (Fig. 3*b*). Average number of cod nets per crew increased for all three generations of gillnetters, roughly doubling from the start to the middle of the career, and doubling again for first-generation fishers (Fig. 3*c*). Total number of cod nets owned by first-generation fishers increased from 50 at the start of their careers to 214 at the end (Fig. 3*d*). Trends for second-generation fishers were similar. Number of cod nets owned by third-generation fishers increased to midcareer and then decreased. Fishers generally attributed this decrease to declining catches from cod nets. Number of traps per crew increased for all three generations, rising by one trap from the start to the middle and rising again from the middle to the end of their careers (Fig. 3*e*). Total number of traps increased throughout the careers of second- and third-generation fishers. In contrast, total number of traps owned by first-generation fishers tended to decline after midcareer (Fig. 3*f*). On most measures, third-generation fishers started out and ended up with higher levels of effort than previous generations.

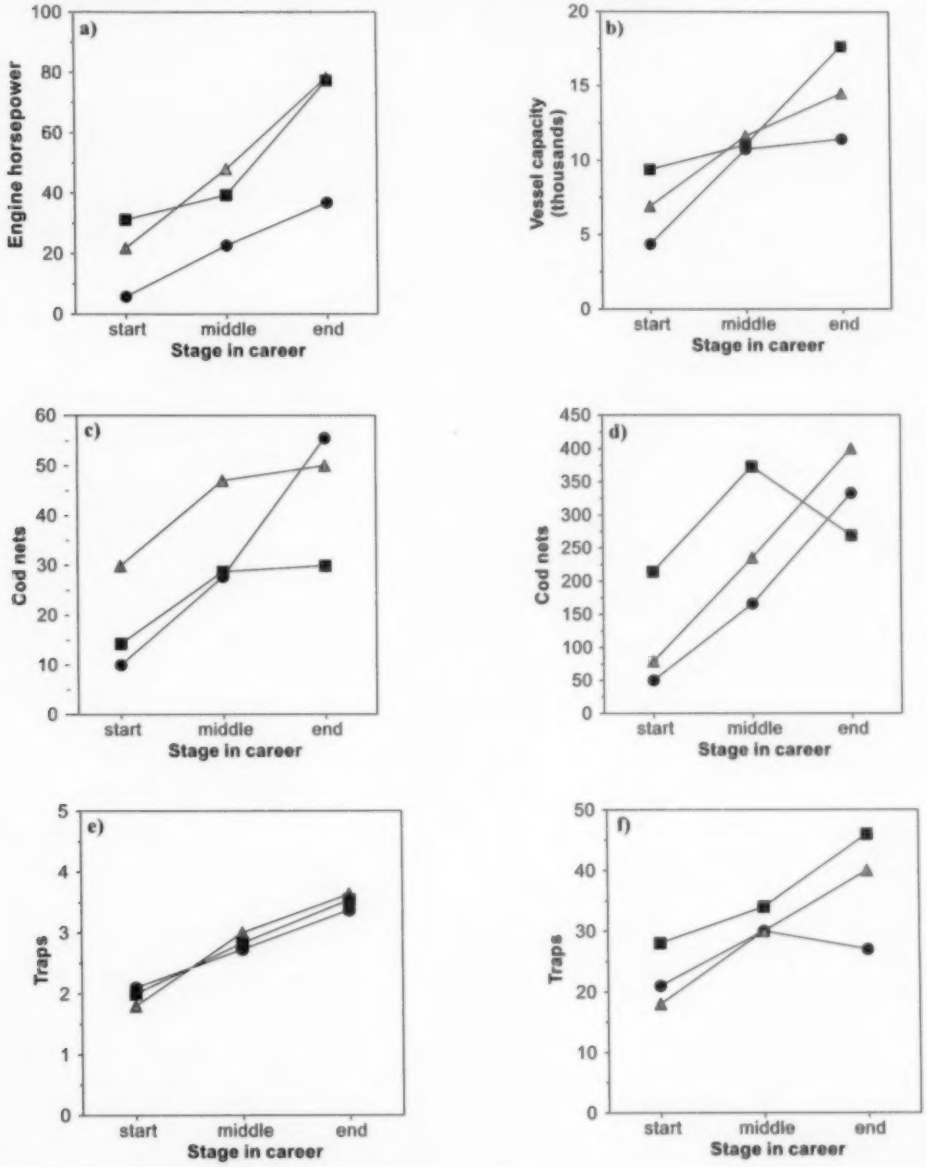
Annual rate of change (percent per year) was plotted against year for engine power, capacity, boat length, and gear. Rate of change in vessel capacity was positive and generally under 20%/year for most fishers before 1980. Capacity increased even more rapidly after 1980 (Fig. 4*a*). Annual rate of increase in engine power accelerated after 1970 for fishers in the early part of their careers as well as for those in the latter part of their careers (Fig. 4*b*). These changes in power and capacity were not reflected in boat length, which on average did not change during the same period for the same fishers (Fig. 4*c*). Annual percent change in number of nets was positive and under 25%/year for most gillnetters (Fig. 4*d*). This reflects the practice of adding a few nets each year. Annual percent change in number of traps was small (under 15%/year) and positive for most fishers (Fig. 4*e*).

Components of effort increased for the inshore fishers interviewed, so it was of interest to find out whether their landings increased or not during a period when landings by the entire inshore sector fluctuated around 200 000 t/year from 1920 to 1992 (Hutchings and Myers 1995). Figure 5 shows reported poor, average, good, and, for 1991, recalled catches for some fishers by year. There was no long-term increase in landings by the sample group, which thus did not differ from the entire inshore fishery in catch trends.

In addition to the move to larger, more powerful vessels and increased gear, a clear spatial shift also occurred in the inshore and nearshore sector. The four skippers with the largest vessels at the time of the moratorium (1992) began

© 1999 NRC Canada

Fig. 3. Generational differences in (*a*) average engine horsepower, (*b*) vessel capacity, (*c*) average number of cod nets per crew, (*d*) total number of cod nets, (*e*) average number of cod traps per crew, and (*f*) total number of cod traps for first-generation (solid circle), second-generation (shaded triangle), and third-generation (solid square) fishers interviewed.



their longliner careers fishing between 8 and 20 mi from their home port. As their boat, horsepower, gear, and capacity increased, their fishing grounds shifted to 50–80 mi offshore and, a few years later, to distances often in excess of 100 mi to an area known as the Virgin Rocks and beyond (Neis et al. 1999).

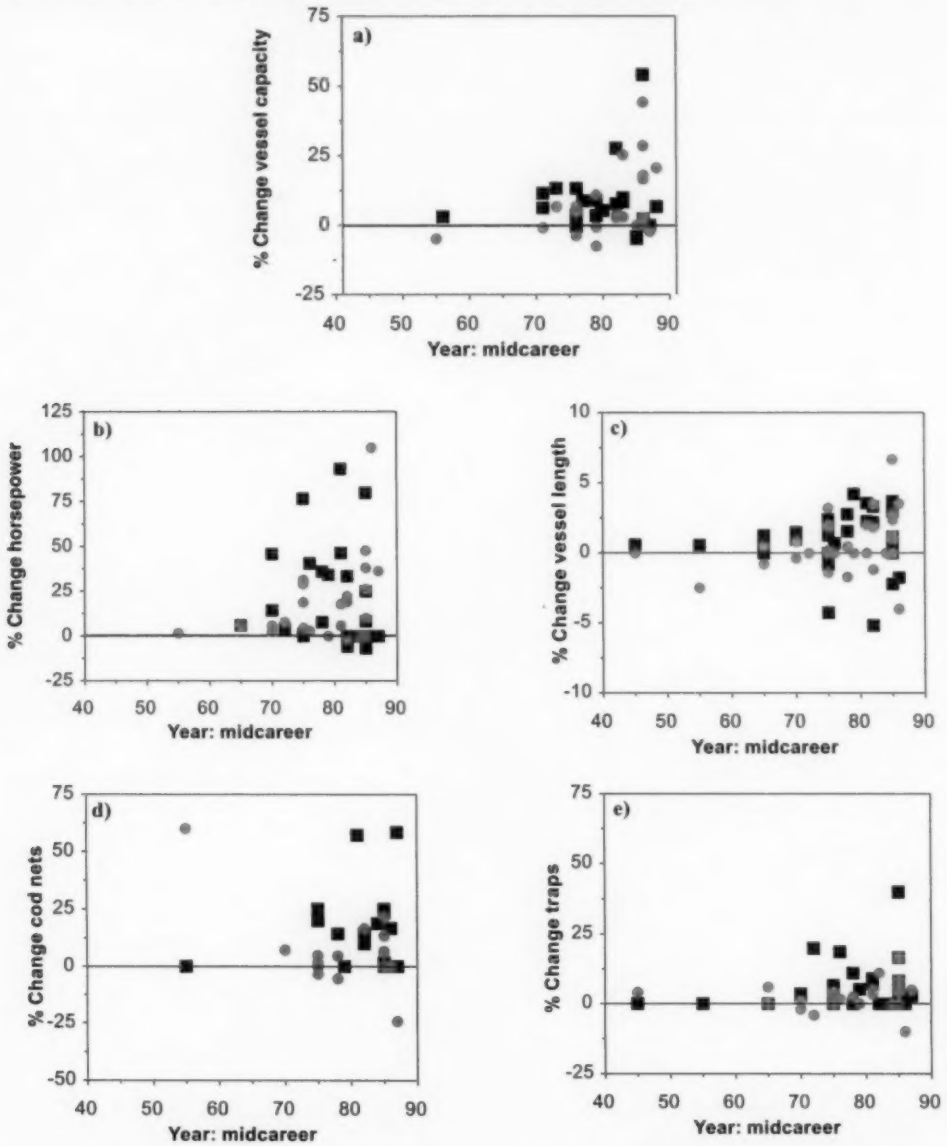
Similar changes in the spatial scale of the lumpfish roe fishery have also occurred. We compared the spatial distribution of fishing in our interview data with that in Mercer (1980). He showed that the lumpfish roe fishery in the study area concentrated exclusively in the area between Southern Bay, Bonavista Bay, and Port Rexton, Trinity Bay. Our interviews identified additional lumpfish fishery locations, so that the fishery now extends down the west coast of Trinity Bay and across to Thornlea and Norman's Cove at the bottom of the bay.

Landings and catch relative to effort

These results suggest that the rate at which fish were intercepted by gear decreased and that catch rates were maintained by increasing other components of effort, including engine power, boat capacity, and amount of gear. For gill

© 1999 NRC Canada

Fig. 4. Percent change per year in (*a*) vessel capacity, (*b*) engine horsepower, (*c*) boat length, (*d*) number of cod nets, and (*e*) number of traps for start to midcareer (solid square) and for midcareer to end of career (shaded circle).



nets, we computed CPUE as catch per net, which was taken as proportional to interception rate (catch per square metre of net) under the assumption of no decrease in panel length or panel height per gill net (a change in catch per gill net can result either from a change in rate of interception (catch pee square metre) or from a change in panel area (square metres per net) due either to longer or to higher panels):

$$\text{CPUE} = \text{catch/net} = (\text{catch/m}^2 \text{ of net}) \times (\text{m}^2 \text{ of net/panel}).$$

The change in CPUE was computed as

$$\Delta\text{CPUE} = (\text{CPUE}_{\text{final}} - \text{CPUE}_{\text{initial}})/\text{CPUE}_{\text{initial}}.$$

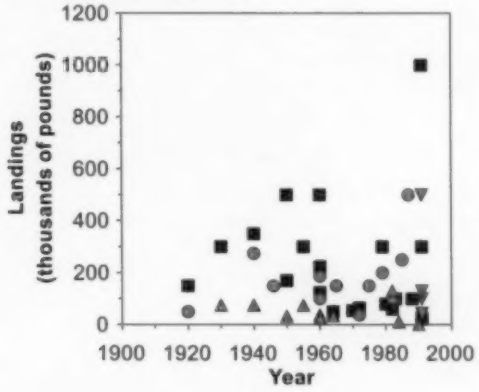
Changes in boat size, horsepower, capacity, and gear amounts were calculated in the same way, as a percentage of the initial value.

Cod fishery

We computed change in CPUE for cod for seven of the nine fishers using longliners (38- to 65-ft boats). Increases in boat length during their career varied from –36 to +32% (Table 1). The single negative value was the result of a longliner skipper abandoning his longliner when confronted with lower catches and longer times away from home to maintain catch rates. Horsepower and capacity increased more than boat length with the exception of the fisher who abandoned

© 1999 NRC Canada

Fig. 5. Poor (shaded upright triangle), average (shaded circle), good (solid square), and 1991 recalled (shaded inverted triangle) catch estimates (pounds) reported by interviewed fishers, vessels <35 ft, 1920s–1990s. Quintals (112 lb of dried cod) converted to 500 lb round weight per quintal.



longlining. Number of nets increased in all cases, with increases varying from 20 to 300% during a career. All seven longliner fishers experienced a reduction in CPUE, with the reductions varying from 14 to 65% (Table 1). Panel length remained at 15.2 m (50 ft), and hence, we attribute change in CPUE to declines in rate of interception.

A similar analysis was undertaken for inshore fishers. Of the five inshore gillnet fishers for whom we could compute percent change in CPUE, all experienced declines that ranged from 48 to 96%. We attribute this to declines in interception rate, in the absence of change in panel length or height.

Change in CPUE was then calculated for trap fishers. CPUE was again computed relative to the area of the gear that intercepts fish, in this case the trap leader (a change in catch rate can result either from a change in rate of interception (catch per square metre of leader) or from a change in leader area (square metres of leader per trap)):

$$\text{CPUE} = \text{catch/trap} = (\text{catch/m}^2 \text{ of leader}) \times (\text{m}^2 \text{ of leader/trap}).$$

Assuming no change in leader area or escape rate, catch rate per trap is proportional to interception rate.

The career-history interviews allowed us to compute percent change in CPUE for seven cod trap fishers. Of these, six experienced a decline in trap CPUE ranging from 13 to 375%. One experienced an increase of 33%. Changes in leader length could not be examined using our data. Escape rate from traps likely decreased during this period due to design changes and more durable netting. Consequently, the observed drops in CPUE likely underestimate the drop in interception rate by these trap fishers.

CPUE declined in the nearshore gillnet fishery pursued by longliners, the inshore gillnet fishery pursued by smaller boats, and the inshore trap fishery. Efficiency increased; hence, we attribute decreases in CPUE to decreases in rate of interception. These decreases in CPUE were not restricted to periods of a few years, and hence, cannot be attributed to temporary declines in availability due to oceanographic conditions or fishing down of strong year-classes in the late 1980s.

Lumpfish roe fishery

Similar declines in CPUE have also occurred in the lumpfish roe fishery. Those fishers that we interviewed who were active in the lumpfish roe fishery (n = 22) unanimously reported that effort, as measured by numbers of fishers and numbers of nets per crew, had increased substantially between the mid-1980s and 1996 (Table 2). Nearly all of these 22 fishers reported an increase in the number of nets that they were using, with percent change ranging up to 900%.

Fishers were also asked to describe changes in their landings of lumpfish roe over time. Fishers who maintained records of their catches were asked to consult these in answering this question. Some checked their receipts, and others relied exclusively on memory. Declines in CPUE ranged from 99% for fisher 23 to 64% for fisher 50 (Table 2). The decline in CPUE, computed as an annual rate, was much greater for recent entrants into the fishery than for fishers who were engaged in the fishery for a decade or more (Fig. 6).

Discussion

This paper demonstrates how personal, career-history interviews, particularly when informed by taxonomy/toponomy interviews and supplemented by followup interviews targeting specific issues, can collect large amounts of information from fishers useful for fisheries assessment. Our findings bear on questions of stock distinctiveness, trends in efficiency in inshore and nearshore fisheries, trends in landings and CPUE, and extending fisheries assessment to include the large amounts of information uncovered by personal interviews.

Stock distinctiveness

The 1992 moratorium on northern cod has highlighted the need to explore the distinctiveness of cod stocks inshore throughout Newfoundland (Hutchings et al. 1993). A significant amount of research on the population structure and migratory and spawning behaviour of these stocks has taken place since 1990, much of it in Bonavista and Trinity bays (e.g., see Hutchings et al. 1993; Rose 1993; Taggart et al. 1994; Wroblewski et al. 1994; Carr et al. 1995; Lilly 1996; Ruzzante et al. 1996, 1997). However, as recently as 1996, the stock status report for 2J3KL cod commented that a major source of uncertainty was related to knowledge concerning "the relationship between inshore cod and fish that used to be widely distributed over the shelf" (Shelton 1996, p. 4). In recent years, an apparent gap between the absence of cod in offshore research vessel surveys and anecdotal and some systematic observations of cod in coastal Newfoundland bays has reinforced commitment to developing a better scientific understanding of different stock components (Rice 1997) and of population dynamics for these stocks.

The recalled observations of fishers reported on here are generally consistent with existing scientific information related to stock structure but provide additional information not readily available in scientific data. Personal interviews show that these inshore and nearshore fishers commonly distinguish between cod that they harvest on the basis of shape,

© 1999 NRC Canada

Table 1. Percent change in efficiency and CPUE between the first and final years in the gillnet fishery, vessels >35 ft.

Fisher	First	Final	Boat length		Capacity		Gear		Catch		CPUE
ID No.	year	year	First	% change	First	% change	First	% change	First	% change	(% change)
27	1979	1992	40	+32	10 000	+700	120	+225	10 000	+30	–60
21	1972	1992	38	+20	9 000	+70	60	+300	10 000	+62	–60
23	1978	1992	38	0	11 000	0	60	+150	9 500	+43	–43
14	1976	1989	42	+19	25 000	+35	100	+300	25 000	+39	–65
24	1967	1992	41	–36	5 500	+16	80	+50	5 500	+27	–15
41	1976	1992	38	+3	11 000	+109	60	+200	11 000	+16	–61
28	1988	1992	40	0	24 000	+90	72	+20	24 000	+3	–14

Table 2. Lumproe catch (pounds) and effort (nets) of fishers interviewed on Bonavista Peninsula, Newfoundland.

Fisher								
ID No.	Year	Initial catch	Nets	Year	Final catch	Nets	ΔNets (%)	ΔCPUE (%)
23	1978	1000/haul	6	1995	100/haul	50	+100	–99
1	1978	1400/haul	20	1994	174/haul	50	+150	–95
48	1980	4500/season	15	1995	1000/season	30	+100	–89
42	1980	500/season	6	1996	200/season	30	+400	–92
37	1983	2500/season	5	1995	1100/season	25	+400	–91
45	1984	450/haul	20	1991		25	+25	
53	1985	200/haul	12	1995	200/haul	40	+233	–70
55	1985	300/week	5	1993	150/week	50	+900	–95
38	1985	15 000/season	20	1995	4000/season	40	+100	–87
32	1986	9000/season	20	1996	2500/season	50	+150	–89
47	1986	200/day	20	1995	100	40	+100	–75
26	1986	750/haul	12	1996	30/haul	37	+208	–99
50	1987	100/week	12	1996	50/week	45	+317	–64
6	1987	100/day	12	1995	100/day	40	+233	–70
3	1988	35 000/season	50	1996	3500/season	50	0	–90
5	1988	300/haul	35	1995	75/haul	50	+43	–82
11	1988	4500/season	32	1996	2000/season	50	+56	–72
42	1990	80/day	30	1995		50	+67	
t6[a]	1990	16 000/season	20	1996	850/season	50	+150	–98
31	1991	280/haul	25	1996	76/haul	25	0	–73
46	1992	4000/season	10	1995	400/season	20	+100	–95
22	1993	60/day	12	1995	60/day	35	+190	–66

Note: Per day, every day; per haul, all nets in 1 day at irregular intervals; CPUE, catch per net. See text for calculation of ΔNets and ΔCPUE.

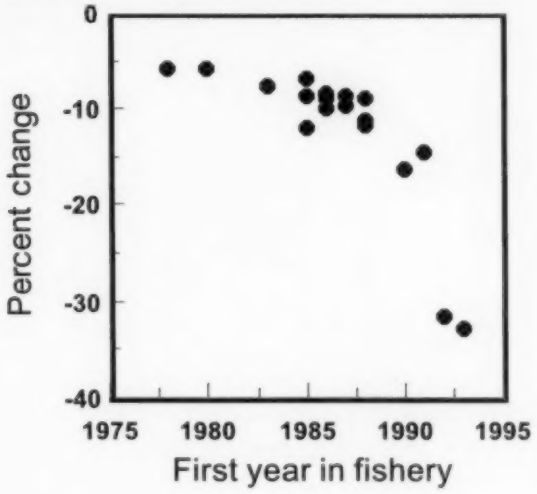
[a]Taxonomy interviews.

diet, colour, size, the depth at which they are fished, whether they are "spawny," and the timing and direction of their movements. A more detailed discussion of these and related findings can be found in Fischer et al. (1997) and Neis (1998). A comparison of interview data from fishers in the Bonavista area and from within the bays, informed by their taxonomic terms for cod, suggests that the temporal and spatial dynamics of the cod fisheries in these two areas differ. Observations from sampled fishers at the bottom of Trinity and Bonavista bays provide strong evidence of observed overwintering cod that is largely absent from interviews done in the Bonavista area. The former fishers have intercepted cod migrating into the arms in the bays in the fall and participated in fall, winter, and spring fisheries for these cod during the 1980s. Differences in observations between fishers within the bays and those in the Bonavista area are consistent with tagging and landings data indicating that fisheries in these areas draw to differing degrees on overwintering cod and cod migrating from offshore with the "capelin fish" (Lilly 1996). Fishers' observations suggest that the spatial and temporal distribution of cod may be related not only to stock structure but also to the size structure of fish populations. They had observed apparent size-related differences in depth and behaviour that have been documented for other species, with many believing that large, old cod settle out in deep trenches in the Bays and observing that these cod differ in terms of colour and behaviour from shoaler water cod (Fischer et al. 1997; Merrett and Haedrich 1997).

It is known that spawning areas for northern cod are discrete, often distant from other spawning areas, and that some are located in the bays. Such disjunct spatial distributions may indicate reproductive isolation among cod populations (Hutchings et al. 1993; Taggart et al. 1994). Interviewed fishers had observed cod in spawning condition, including some possible spawning aggregations, at several locations in Trinity and Bonavista bays. Whereas some of these spawn-

© 1999 NRC Canada

Fig. 6. Percent change in CPUE per annum in the lumpfish roe fishery of interviewed fishers, 1978–1996.



ing aggregations have already been scientifically documented, often with assistance from fishers, others have not and should be investigated. In some cases, these spawning aggregations may no longer exist.

In New England, interviews with older, retired fishers have produced maps of present and former spawning areas for cod and haddock. These interviews also generated information on the sequence and nature of the collapse of local stocks, highlighting localized fishing impacts (Ames 1998). Similarly, Trinity and Bonavista Bay fishers who had gillnetted very large, whitish cod in the deeper areas of the bays believed that these local aggregations of mother fish disappeared with the expansion of the gillnet fisheries in the 1960s and 1970s. They also observed juvenile cod, making associations between fluctuations in juvenile cod abundance and a large bycatch of juvenile cod in capelin traps in Trinity Bay (M. Morris, Memorial University of Newfoundland, unpublished data).

Efficiency

Fishers described a variety of innovations that are relevant to an assessment of changes in efficiency q_{comm}. Interviews showed that two components of q_{comm}, capacity and horsepower of inshore vessels, increased slowly in the 1960s, more rapidly in the 1970s, and still more rapidly in the 1980s. The number of traps per person increased steadily in the 1970s and 1980s and there were significant changes in mesh size and design. After 1980, the number of cod traps owned by the sampled fishers increased from 33 to 49 traps (n = 15). Cod nets per boat increased in the 1970s more than afterward. The number of gill nets owned by the sampled fishers increased from 1480 nets in 1980 to 2900 in 1991 (n = 25). Landings per boat fluctuated, with no long-term increase for the group interviewed. During this period, capacity, engine power, and gear amount increased in both the inshore and nearshore fisheries.

The spatial scales of the trap fishery and gillnet fisheries also increased. Some fishers at the bottom of Trinity Bay moved to St. Mary's Bay to trap cod in the late 1980s and early 1990s, and some from the middle of the bay (New Bonaventure area) moved to Labrador in 1987. Fishers from within Bonavista Bay are reported to have fished in the Bonavista area in the year or two prior to the moratorium. Hutchings and Myers (1994) identified important spatial changes in the inshore gillnet fishery, which began extending offshore onto new grounds as effort increased and catch per net declined (Fig. 7). Interviews with four gillnet fishers on longliners in the Bonavista–Catalina areas showed similar shifts in spatial scale accompanied by movements to larger boats, more gear, and more frequent hauling of that gear (Neis et al. 1999). They showed that these movements were a consequence of declining catches closer to shore and in the Bonavista area. Fishers said that they had to fish further afield and further offshore in order to maintain their catch rates.

Fishers described a number of other innovations that contributed to efficiency. The interviews and subsequent public meetings suggest that innovation that increases efficiency is driven by multiple factors. Lower resource abundance is one important factor, with fishers reporting fewer and generally smaller fish after gillnet grounds had been fished for a few years and pressure to change gear design in the inshore trap fishery and the spatial scale of their fishing effort. Another factor is the need to provide for greater comfort and safety as the spatial scale of fishing expands. Another factor might be termed a "colleague effect," which refers to fishers feeling peer pressure to keep up with their colleagues in the move to bigger boats even if they do not fully feel a need to increase capacity and gear. A number of fishers mentioned the role of governments in encouraging the trend to larger boats through various loan and subsidy programs.

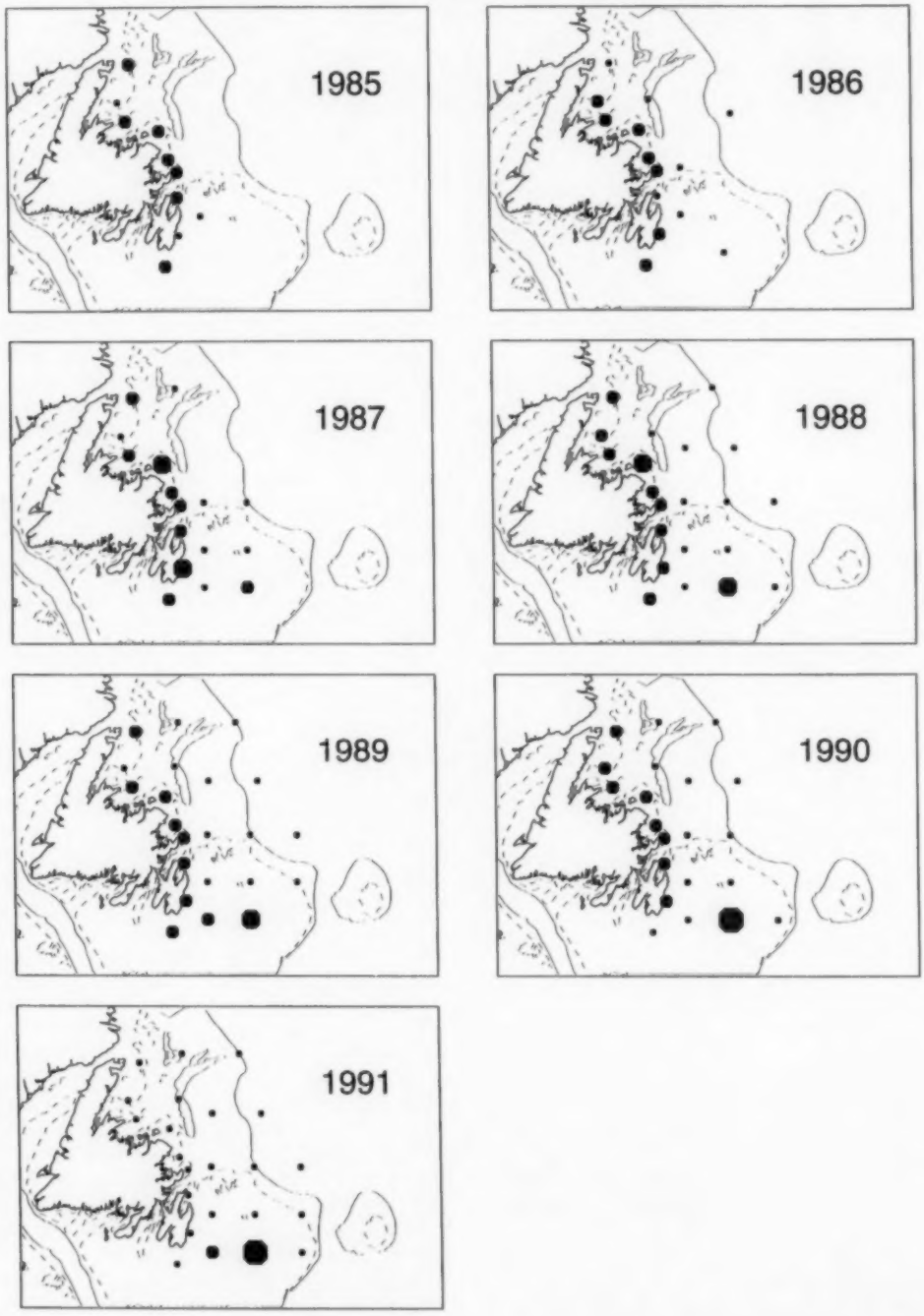
Landings and CPUE

For cod, results from interviewing resource users were consistent with results from analysis of purchase slip data. Spatial changes in gillnet fishing effort in the latter half of the 1980s and early 1990s are evident from purchase slip records compiled by the Statistical Coordinating Committee for the Atlantic Coast (DFO, Ottawa, Ont.). These data, which report the Northwest Atlantic Fisheries Organization (NAFO) unit areas in 3L from which cod were captured, reveal a significant offshore expansion of this fixed-gear fishery based on purchase slip data, suggesting that CPUE for cod in the inshore had begun to decline as early as the mid-1980s (Fig. 7). This decline could, in theory, have been the result of fishing out of a few strong year-classes, causing a short-term reduction in CPUE. Results from interviewing resource users show, however, that declines in CPUE began before 1980 and that these were associated with increases in boat capacity, engine power, and gear fished per boat. Declines in CPUE for the inshore cod fishery occurred over decades and cannot be attributed to short-term drops in stock size due to recruitment variability.

For lumpfish, a recent DFO study of CPUE produced results consistent with our findings that CPUE declined drastically in the lumpfish roe fishery in the 1990s (Myers et al. 1995). Another study based on interviews with fishers in Bonavista and Notre Dame bays produced similar results (Potter 1996). Limited historical data also support the con-

© 1999 NRC Canada

Fig. 7. Spatial distribution of gillnet catches of northern cod between 1985 and 1991. The four sizes of symbols, beginning with the smallest, represent catches of 1–100, 100–1000, 1000 – 10 000, and >10 000 t (to a maximum of 18 000 t). Depth contours are the 200- and 400-m isobaths. Each of the circles is positioned in the centre of the NAFO unit area from which the catches were reportedly taken (NAFO unit areas represented here include 3Ka to 3Ki, 3La to 3Lj, and 3Ls to 3Lr).



clusion that CPUE has declined dramatically since the 1970s. According to Mercer (1980), there were an estimated 50 crews fishing lumpfish in the Bonavista Peninsula region in 1979. They fished between three and 30 nets each for an average of 16.5 nets/crew. Landings in the region for 1979 totalled 85 467 kg for an average of 104 kg·net^{-1}·season^{-1}.

© 1999 NRC Canada

In the period between 1994 and 1996, an average of 432 fishers were licensed to fish for lumpfish roe in the same region. Based on the practices of our sample of fishers, we estimate they would have fished an average of 40 nets/vessel. With an estimated crew size of two, this would total 8748 nets. Landings in the region in 1995 were 82 379 kg for an estimated CPUE of 9.4 $kg \cdot net^{-1} \cdot season^{-1}$, indicating a reduction of 91% in CPUE between 1980 and 1995.

Interview data indicate that lumpfish catch rates in the study area may have declined by as much as 90% between 1980 and 1996 (Table 2), despite an increase in the spatial scale of this fishery from a concentration in the Bonavista area to most of the coastal area including areas of marginal lumpfish habitat. The data also indicate that the rate of decline in catch rates has accelerated in recent years (Fig. 6). Decreases in catch rates by passive gear reflect decreases in encounter rate between fish and gear. Such decreases can result from decreases in fish density, decreases in movement, or decreases in efficacy of capture. Decreases in movement at the scale of a decade have not, to our knowledge, been reported in any species of fish. Decrease in efficacy of capture has not been reported and would be surprising, given the increases in skill that typically occur within fisheries. Decreases in density can result either from a decrease in numbers or from an increase in area occupied. In species that establish territories during spawning, such as lumpfish, decreases in density due to increase in area occupied are unlikely because of competition for better sites. Because the changes that we observed occurred at the scale of hundreds of kilometres, we conclude that decreases in catch rates were due to decreases in population size. The fishers that we interviewed attributed the decline in lumpfish to the combined effects of increased numbers of fishers, increased numbers of nets, and the destructive effects of a roe fishery in which the females are killed for their eggs.

The lumpfish roe fishery was started in this area in 1969. Much of the existing Newfoundland-based research on lumpfish biology and CPUE in this fishery dates from provincial government studies carried out between 1978 and 1982 in association with experiments with fishing in new areas and with new gear (Mercer 1980; Blackwood 1982). Provincial researchers recommended that DFO carry out the scientific research necessary for management of this fishery. However, little additional research on lumpfish ecology, migration, and trends in size and catch rates was carried out during the subsequent decade. As of 1996, commercial CPUE data were extremely limited (Stevenson and Baird 1988). The first lumpfish management plan was not introduced until 1994 and the spatial scale of the fishery had not been monitored. These gaps have made it difficult for DFO to establish trends in lumpfish catch rates, age and length composition, sex ratio, and spatial scale occupied. In a fishery such as this, information assembled from structured interviews can provide useful information on trends.

Extending stock assessment to include resource users

Personal interviews, using a career-history approach, that are informed by awareness of the fish and place names used by fishers uncovered large amounts of information. When assembled, the information brought to light trends that are consistent across many fishers, across gear sectors, and across slight differences in the format of individual interviews. These trends are valid on an ordinal scale ("gear efficiency increased over decades") even if they cannot be applied on a ratio scale ("gear efficiency in the population increased by x%"). Our efforts to compute change in CPUE in the trap cod and lumpfish roe fishery showed that detail will be lost in a broad-ranging career-history interview but can be acquired, checked, or updated in short, relatively inexpensive followup telephone interviews. Our research also revealed that some fishers have maintained catch receipts and logbooks from earlier points in their careers. These alternative sources, which come to light during personal interviews, should ideally be consulted during interviews, but a return visit may be required. Fishers can also be urged to consult their records during a telephone followup. Reliability and internal consistency are increased by comparing results from different techniques (triangulation), by internal comparisons within and between transcripts, and with the results of feedback meetings and independent interviews and discussions with fishers, such as those held in Melrose and Chance Cove (Fischer et al. 1997).

Interviews can be used to provide information on long-term trends in efficiency, independent of catch rates. Often, calibration methods, based on regression of commercial versus research vessel catches, cannot be used for the inshore sector because of the absence of research vessel information for this area. Interviews could also be used to provide information and changes in efficiency at time scales of a year, more timely than information that arises from calibration methods. This could be accomplished by reinterviewing a group of index fishers each year. Information on boat capacity, engine power, and gear could be updated annually to provide measures of change in efficiency that are independent of stock size of landings. These measures are both independent and timely, and hence, more useful than calibration-based measures that assume commercial and research fishing on the same stock and that require several years to detect trends in efficiency.

Interviews could also be used to provide timely information on changes in terminal mortality. Assessment methods based on population reconstruction (e.g., virtual population analysis) are highly sensitive to errors in terminal mortality (Hilborn and Walters 1992). Terminal mortality from research vessel surveys will be imprecise because of the small number of fish caught and may be inaccurate (biased downward) for the same reason. A check on this, using independent data from interviews of resource users, is needed.

Fishers' knowledge of stocks is primarily acquired to optimize catches while minimizing effort. It is largely based on observations that take place on their fishing grounds during the fishing season. Individual knowledge thus tends to be localized and seasonal. We have shown that a sampling strategy that spreads interviews throughout a region and across sectors (<35 and >35 ft vessels) can permit the aggregation of local information to arrive at a regional perspective. In addition, the seasonality of fishers' observations can be minimized in career-history interviews that include information on fisheries for a range of species using a broad range of gears. Fishers tend to closely observe those environmental features that are linked to fishing success, such seasonal movements, habitat preferences, feeding behaviour, and

© 1999 NRC Canada

abundance dynamics, and those physical attributes of fishing grounds that affect fish distribution, the performance of gear, and fishing time, such as wind direction, currents, water temperature and clarity, bottom characteristics, and local assemblage structures (related to bycatch and discarding) as well as gear fouling (i.e., slub on the nets). Their observations can have a temporal scale of as long as 40 or 50 years for older fishers and, as in the case of the lumpfish roe fishery, often include information on species and fisheries that has been poorly monitored in the past.

Finding ways to make comparisons between fishers' observations and data drawn from more traditional scientific sources could improve the potential for more informed and more accepted decisions on stock status and management. Personal interviews with a career-history format can generate a baseline of information for a particular fisher and, when data are aggregated, for local and regional fisheries where little scientific data exist. They can also provide the basis for the development of a relationship of trust, so-called "rapport" (Punch 1986). Such interviews need to be conducted in person rather than by mail or over the telephone. Where mail and telephone interviews are not preceded by a detailed, face-to-face interview, they can produce partial or flawed conclusions and can convey the message that assessment scientists are not interested in the results.

Systematic and careful collection of information from fishers as part of a comanagement regime could substantially improve the knowledge base for fisheries management and, relatedly, fisher compliance in the future (Pinkerton 1994). Resource users develop a detailed, small-scale understanding of population complexes, while scientific management typically aims at a larger scale. This mismatch in spatial scale can lead to different assessments of stock status and apparent disagreement where none may exist. Scientific models of population structure should be consistent with fishers' observations of spawning locations, predictable seasonal movements, and morphological differences. Fishers' observations can also help us interpret spatial and temporal variation in catch data, such as those contained in purchase slip data bases (Lilly 1996). Disagreements between local and larger scale perceptions of stock status indicate the need for nested assessments at different spatial scales (e.g., local seasonal, regional annual) and for multiple methodologies. Resource users are unlikely to assent to a statement of large-scale status that conflicts with their own direct experience but may be more likely to assent when larger scale understandings are compared through a composite of localized observations from fishers throughout a region.

Acknowledgements

This research was funded by a 3-year Strategic Science and Technology grant from the Social Science and Humanities Research Council and supported by Tricouncil Eco-Research Programme grant 334-884. We are grateful for this support. We thank Melanie Morris for technical support. We would also like to thank Jake Rice, Joanne Morgan, and five anonymous referees for comments on earlier versions of the manuscript.

References

Ames, T. 1998. Cod and haddock spawning grounds in the Gulf of Maine from Grand Manan Channel to Ipswich Bay. *In* The implications of localized fishery stocks. *Edited by* H. von Herbing, I.I. Kornfield, M. Tupper, and J. Wilson. NRAES, Ithaca, N.Y. pp. 55–64.

Babbie, E. 1989. The practice of social research. 5th ed. Wadsworth, Belmont, Calif.

Berkes, F. 1993. Traditional ecological knowledge in perspective. *In* Traditional ecological knowledge: concepts and cases. *Edited by* Julian T. Inglis. International Development Research Centre, Ottawa, Ont. pp. 1–10.

Blackwood, G. 1982. Contributions to the biology of the lumpfish *Cyclopterus lumpus* L. 1758 (Cyclopteridae) with reference to the Newfoundland lumpfish roe fishery. Honours dissertation, Department of Biology, Memorial University of Newfoundland, St. John's, Nfld.

Carr, S.M., Snellen, A.J., Howse, K.A., and Wroblewski, J.S. 1995. Mitochondrial DNA sequence variation and genetic stock structure of Atlantic cod (*Gadus morhua*) from bay and offshore locations on the Newfoundland continental shelf. Mol. Ecol. **4**: 79–88.

Evans, G. (*Editor*). 1996. Meetings to review assessments of groundfish stocks in the Newfoundland region. Canadian Stock Assessment Proceedings Series 96/7. Department of Fisheries and Oceans, Science Branch, St. John's, Nfld.

Fischer, J., Brace, A., Clarke, R., Donovan, W., Humby, E., Humby, P., McNamara, W., Peters, B., Rowe, L., Smith, E., and Smith, J. 1997. What you always wanted to know about fish in Trinity Bay and never dared to ask. Occasional Paper, Eco-Research Project, Memorial University, St. John's, Nfld.

Gunn, A., Arlooktoo, G., and Kaomayok, D. 1988. The contribution of the ecological knowledge of Inuit to wildlife management in the Northwest Territories. *In* Traditional knowledge and renewable resource management in northern regions. *Edited by* M.R. Freeman and L. Carbyn. Boreal Institute for Northern Studies, Edmonton, Alta. pp. 22–30.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

Hutchings, J.A. 1996. Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. Can. J. Fish. Aquat. Sci. **53**: 943–962.

Hutchings, J.A., and Myers, R.A. 1994. What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. **51**: 2126–2146.

Hutchings, J.A., and Myers, R.A. 1995. The biological collapse of Atlantic cod off Newfoundland and Labrador. The North Atlantic fisheries: successes, failures and challenges. *Edited by* R. Arnason and L. Felt. Institute of Island Studies, Charlottetown, P.E.I. pp. 37–94.

Hutchings, J.A., Myers, R.A., and Lilly, G.R. 1993. Geographic variation in the spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **50**: 2457–2467.

Hutchings, J.A., Neis, B., and Ripley, P. 1997. The "nature of cod": perceptions of stock structure and cod behaviour by fishermen, "experts" and scientists from the nineteenth century to the present. *In* Marine resources and human societies in the North Atlantic since 1500. *Edited by* D. Vickers. Conf. Pap. 5. Institute of Social and Economic Research, St. John's, Nfld. pp. 123–188.

Johannes, R.E. 1993. Integrating traditional ecological knowledge and management with environmental assessment. *In* Traditional

© 1999 NRC Canada

knowledge: concepts and cases. *Edited by* J.T. Inglis. International Development Research Centre, Ottawa, Ont. pp. 33–40.

Lear, W.H. 1984. Discrimination of the stock complex of Atlantic cod (*Gadus morhua*) off southern Labrador and eastern Newfoundland, as inferred from tagging studies. J. Northwest Atl. Fish. Sci. **5**: 143–159.

Lilly, G.R. 1996. Observations on cod in the inshore environment of eastern Newfoundland. NAFO SCR. Doc. 96/59.

Mailhot, J. 1993. Traditional ecological knowledge: the diversity of knowledge systems and their study. The Great Whale Public Review Support Office, Montreal, Que.

Mercer, K.M. 1980. Lumpfish roe fishery development in Newfoundland during 1980. Government of Newfoundland and Labrador, St. John's, Nfld.

Merrett, N.R., and Haedrich, R.L. 1997. Deep-sea demersal fish and fisheries. Chapman and Hall, London, U.K.

Myers, R.A., Sjare, B., and Luscombe, R.B. 1995. An analysis of lumpfish from data on individual fishermen in the Newfoundland region. DFO Atl. Fish. Res. Doc. 95/66. Department of Fisheries and Oceans, Science Branch, St. John's, Nfld.

Neis, B. 1998. Fishers' ecological knowledge and the identification and management of localized populations of Atlantic cod (*Gadus morhua*) in northeast Newfoundland. *In* The implications of localized fishery stocks. *Edited by* H. von Herbing, I.I. Kornfield, M. Tupper, and J. Wilson. NRAES, Ithaca, N.Y. pp. 177–188.

Neis, B., Felt, L., Haedrich, R.L., and Schneider, D.C. 1999. An interdisciplinary methodology for collecting and integrating fishers' ecological knowledge into resource management. *In* Fishing places, fishing people: issues and traditions in Canadian small-scale fisheries. *Edited by* D. Newell and R. Ommer. University of Toronto Press, Toronto, Ont. pp. 217–238.

Pinkerton, E. 1994. Local fisheries co-management: a review of international experiences and their implications for salmon management in British Columbia. Can. J. Fish. Aquat. Sci. **51**: 1–17.

Pope, J.G. 1977. Estimation of fishing mortality, its precision and implications for the managment of fisheries. *In* Fisheries mathematics. *Edited by* J.H. Steele. Academic Press, London, U.K. pp. 63–76.

Potter, A.J. 1996. Identification of inshore spawning areas: potential marine protected areas? MARA 5002 Graduate Project, Marine Affairs Program, Dalhousie University, Halifax, N.S.

Punch, M. 1986. The politics and ethics of fieldwork. Sage, Beverly Hills, Calif.

Rice, J.R. (*Editor*). 1997. Proceedings of the Workshop on Inshore–Offshore Stock Components. Canadian Stock Assessment Proceedings Series 97/06. Department of Fisheries and Oceans, Canadian Stock Assessment Secretariat, Ottawa, Ont.

Rose, G.A. 1993. Cod spawning on a migration highway in the north-west Atlantic. Nature (Lond.), **366**: 458–461.

Rose, G.A. 1996. Cross-shelf migrations of cod in NAFO divisions 2J3KL in May and June 1995: some preliminary findings of a longer term study. NAFO SCR Doc. 96/57.

Ruzzante, D.E., Taggart, C.T., Cook, D., and Goddard, S.V. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. **53**: 634–645.

Ruzzante, D.E., Taggart, C.T., Cook, D., and Goddard, S.V. 1997. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: a test, and evidence of temporal stability. Can. J. Fish. Aquat. Sci. **54**: 2700–2708.

Shelton, P. 1996. Northern (2J3KL) cod. DFO Atl. Fish. Stock Status Rep. SSR 96/45.

Smedbol, R.K., and Wroblewski, J.S. 1997. Evidence for inshore spawning of northern Atlantic cod (*Gadus morhua*) in Trinity Bay, Newfoundland, 1991–1993. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 177–186.

Smith, T.D. 1994. Scaling fisheries: the science of measuring the effects of fishing, 1855–1955. Cambridge University Press, New York.

Stephenson, R.L. 1998. Consideration of localized stocks in management: a case statement and a case study. *In* The implications of localized fishery stocks. *Edited by* H. von Herbing, I.I. Kornfield, M. Tupper, and J. Wilson. NRAES, Ithaca, N.Y. pp. 160–168.

Stevenson, S.C., and Baird, J.W. 1988. The fishery for lumpfish (*Cyclopterus lumpus*) in Newfoundland waters. Can. Tech. Rep. Fish. Aquat. Sci. No. 1595.

Taggart, C.T., and Frank, K. 1987. Coastal upwelling and *Oikopleura* occurrence ("slub"): a model and potential application to inshore fisheries. Can. J. Fish. Aquat. Sci. **44**: 1729–1736.

Taggart, C.T., Anderson, J., Bishop, C., Colbourne, E., Hutchings, J., Lilly, G., Morgan, J., Murphy, E., Myers, R., Rose, G., and Shelton, P. 1994. Overview of cod stocks, biology, and environment in the northwest Atlantic region of Newfoundland with emphasis on northern cod. ICES Mar. Sci. Symp. **198**: 147–157.

Taggart, C.T., Penney, P., Barrowman, N., and George, C. 1995. The 1954–1993 Newfoundland cod-tagging database: statistical summaries and spatial–temporal distributions. Can. Tech. Rep. Fish. Aquat. Sci. No. 2042.

Wroblewski, J.S., Bailey, W.L., and Howse, K.A. 1994. Observations of adult Atlantic cod (*Gadus morhua*) overwintering in nearshore waters of Trinity Bay, Newfoundland. Can. J. Fish. Aquat. Sci. **51**: 142–150.

© 1999 NRC Canada

An individual-based model of pigment flux in lakes: implications for organic biogeochemistry and paleoecology

K. Cuddington and P.R. Leavitt

Abstract: Vertical fluxes of pigments are used in limnology to monitor phytoplankton abundance, herbivore grazing, ecosystem efficiency, and historical changes in production. However, significant pigment degradation can occur during algal sedimentation. We used an individual-based model of pigment flux to quantify the relative importance of production and degradation as controls of pigment sedimentation. Pigment deposition increased with production, sinking rate, and phytoplankton depth and declined as lake depth and the depth of oxygen penetration increased. Unexpectedly, pigment sedimentation rate was not sensitive to variation in photooxidation rates, even though bleaching accounted for the second greatest amount of pigment loss. Digestion by zooplankton caused the most pigment degradation, but grazing increased pigment deposition when digestive losses were less than those due to oxidation of pigments in ungrazed cells. The model suggests that algal production may be underestimated in sedimentation studies that do not consider variability in water column depth. Further, comparisons with paleoecological analyses suggest that some inferred increases in production during lake ontogeny may arise from changes in regulation of pigment fluxes rather than from increased algal production.

Résumé : Les flux verticaux de pigments servent en limnologie à surveiller l'abondance du phytoplancton, le broutage par les herbivores, l'efficience de l'écosystème et les changements historiques dans la production. Toutefois, une dégradation importante des pigments peut se produire pendant la sédimentation des algues. Nous nous sommes servis d'un modèle individualisé des flux de pigments pour quantifier l'importance relative de la production et de la dégradation comme contrôles de la sédimentation des pigments. Le dépôt de pigments augmentait avec la production, le rythme de descente et la profondeur du phytoplancton, et baissait à mesure qu'augmentaient la profondeur du lac et la profondeur de pénétration de l'oxygène. De façon imprévue, le taux de sédimentation des pigments n'était pas sensible aux variations des taux de photo-oxydation, même si la décoloration constituait le deuxième facteur de perte de pigments, le premier étant la digestion par le zooplancton; toutefois, le broutage accroissait le dépôt de pigments quand les pertes digestives étaient inférieures à celles qui étaient dues à l'oxydation dans les cellules non broutées. Le modèle permet de penser que la production algale est sous-estimée dans les études de sédimentation qui ne tiennent pas compte de la variabilité de la profondeur dans la colonne d'eau. En outre, les comparaisons avec les analyses paléoécologiques indiquent que certaines augmentations supposées de la production pendant l'ontogénie des lacs pourraient être causées par des changements dans la régulation des flux de pigments plutôt que par une hausse de la production algale.

[Traduit par la Rédaction]

Introduction

A better understanding of the factors controlling algal pigment deposition is required to confidently use the distribution and concentration of these pigments in lake sediments for biogeochemical or paleolimnological studies. Chlorophylls and carotenoids have been used to monitor changes in productivity and community composition (Millie et al. 1993; Claustre 1994), vertical zonation of algae and bacteria (Hurley and Watras 1992; Hurley and Garrison 1993), plankton sedimentation (Poister et al. 1999), herbivore grazing (Welschmeyer and Lorenzen 1985*a*, 1985*b*; Carpenter et al. 1988), and ecosystem efficiency (Baines and Pace 1994). Production, algal biomass, and pigment sedimentation rates are correlated (Gorham et al. 1974; Guilizzoni et al. 1983; Leavitt and Findlay 1994), but the relationship is often weak, possibly because of lake-specific variation in pigment loss during deposition (Carpenter et al. 1988; Hurley and Armstrong 1991; Steenbergen et al. 1994).

The actual amount of pigment delivered to the sediments may depend on the losses or modifications due to chemical oxidation (Leavitt and Carpenter 1990*a*), photooxidation (Welschmeyer and Lorenzen 1985*a*), microbially meditated decomposition (Bianchi et al. 1993), and losses during herbivore digestive processes (Head and Harris 1992). Once deposited, pigments can be further degraded by rapid chemical or biological processes (Repeta and Gagosian 1984; Hurley and Armstrong 1991; Steenbergen et al. 1994; Sinninghe

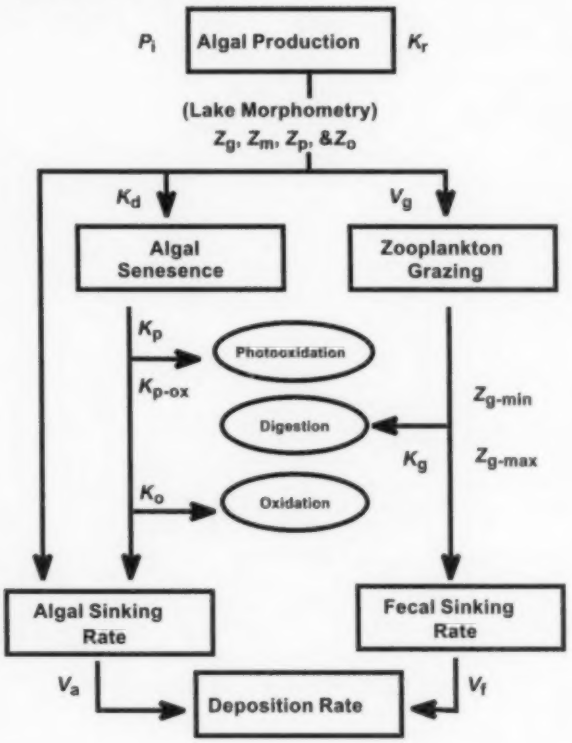
Received September 8, 1998. Accepted June 10, 1999. J14780

K. Cuddington[1] and P.R. Leavitt. Limnology Laboratory, Department of Biology, University of Regina, Regina, SK S4S 0A2, Canada.

[1]Author for all correspondence at the following address: Department of Ecology and Evolutionary Biology, University of Connecticut, 75 N. Eagleville Road, U-43, Storrs, CT 06269-3043, U.S.A.

© 1999 NRC Canada

Fig. 1. Schematic diagram of a model of autochthonous pigment flux in lakes. Abbreviations for rate coefficients are presented in Table 1 and the text.



Damste and Koopmans 1997). However, the magnitude and relative importance of variation in these loss factors may change with the specific lake depth, the penetration depths of light and oxygen relative to lake depth, and the mean depth of the producer population relative to all three factors (Hurley and Garrison 1993; Baines and Pace 1994). As well, deposition of pigments can be altered independently of primary production by changes in the composition and size structure of the food web (e.g., Welschmeyer and Lorenzen 1985*a*; Carpenter et al. 1988).

Mass balance budgets and mesocosm experiments have been used to identify the important factors that influence pigment sedimentation, but regulation of material flux is difficult to quantify with experiments, and empirical studies often support apparently contradictory hypotheses. For example, observational studies suggest that pigment deposition is correlated with total algal biomass (Gorham et al. 1974; Guilizzoni et al. 1983), the abundance of metalimnetic phytoplankton (Hurley and Garrison 1993; Baines and Pace 1994), sinking of large cells (Poister et al. 1999), or grazing by large-bodied herbivores (Welschmeyer and Lorenzen 1985*b*; Carpenter et al. 1986). Although direct correspondence between euphotic zone algal standing crop and pigment deposition has been recently demonstrated (Leavitt and Findlay 1994; Leavitt et al. 1999), it has proven difficult to establish the importance of herbivores and metalimnetic blooms in regulating pigment deposition (e.g., Poister et al. 1999). Comparisons between lakes, as well as the examination of lengthy sedimentation records from a single lake, may be complicated by differences or changes in morphological features such as lake depth. As yet, no study has attempted to determine experimentally how regulation of pigment sedimentation by these diverse mechanisms varies among lakes, possibly because it is impractical to independently manipulate factors that regulate pigment fluxes.

In light of experimental difficulties, it has been suggested that models may be useful in examining biogeochemical fluxes in environments that are not amenable to direct manipulation (Aksnes and Wassman 1993; Sarnelle 1999). To address these issues, we created an individual-based simulation model of algal cells to estimate the relative importance of various factors influencing the rate of pigment deposition. These models are particularly useful when the processes experienced by individuals are dependent on their unique status or location and when particular mechanisms are best represented as a combination of deterministic (e.g., sinking, bleaching) and probabilistic events (e.g., recruitment, grazing, death). Individual-based, or Lagrangian, models (Huston et al. 1988; DeAngelis and Gross 1992) explicitly record the characteristics of each individual and simulate the effects that they experience. This type of model has been successfully used in other studies of plankton biology (e.g., Yamazaki et al. 1991).

Detailed analyses of the model predictions were used to identify potential interactions among regulatory processes that may produce the seemingly contradictory patterns recorded in empirical studies. In conducting these computer experiments, our main objectives were to (*i*) determine if pigment production and deposition remain correlated over a range of conditions despite significant pigment degradation, (*ii*) estimate the relative sensitivity of pigment flux to variation in various physical and biological factors, (*iii*) quantify the relative magnitude of photooxidation, oxidation, and herbivory as degradative processes, (*iv*) determine the effect of mean depth of phytoplankton on pigment deposition rates, (*v*) determine the effect of herbivory on pigment deposition, and (*vi*) determine the effect of lake infilling on pigment deposition rates. Together, these analyses were intended to provide a theoretical framework for future studies of organic matter sedimentation and paleoecological analyses with labile fossils (e.g., pigments, fatty acids).

Model description

Mesocosm experiments, empirical studies, and whole-lake manipulations suggest that pigment deposition is controlled by both biological processes and physical characteristics of the ecosystem. We developed a model of individual algal cells to simulate biological processes including algal recruitment (K_r) and death (K_d), photooxidation ($K_{p\text{-}ox}$), oxidation (K_o), herbivore grazing (V_g), pigment digestion (K_g), algal sinking (V_a), and fecal sinking (V_f) (Fig. 1). This technique allows us to quantify the effect of spatial and temporal variation in regulatory processes by modelling individual pigment-containing phytoplankton. The computer simulation monitored the status of pigment in individual algal cells as the cells descended through a one-dimensional water column, underwent various loss processes, and ultimately were de-

© 1999 NRC Canada

Table 1. Mean and range (generated for 20% CV) of parameter values used in the model simulations and range of parameter values (99% confidence intervals) estimated from empirical studies.

Parameter	Mean value	Modelled range	Observed range	Source(s)*
Biological				
Oxidation coefficient (K_o, day^{-1})	0.058	0.026–0.090	0.0395–0.0929	1, 2, 3, 4
Photooxidation coefficient ($K_{o\text{-}ox}$, E^{-1}·day^{-1})	0.050	0.023–0.077	0.0408–0.0794	5, 6
Grazing degradation (K_g, %·ingestion^{-1})	75	37.0–100.0	54.3–78.8	7
Grazing rate (V_g, %·day^{-1})	20	0.0–58.0	6.3–38.3	8, 9
Feces sinking rate (V_f, m·day^{-1})	4.0	1.80–6.20	1.87–15.1	10
Phytoplankton sinking rate (V_a, m·day^{-1})	0.50	0.23–0.77	0.4768–1.011	5, 8, 9, 11
Phytoplankton recruitment (K_r, %·day^{-1})	10	0.50–53.0	29.0–48.0	11, 12, 13
Initial algal abundance (P_i, cells)	750	340–991	—	
Phytoplankton death (K_d, %·day^{-1})	10	0.40–52.0	0.0–29.0	11, 12
Minimum grazing depth ($Z_{g\text{-}min}$, m)	0.0001	$5.0 \times 10^{-5} - 1.5 \times 10^{-4}$	—	
Maximum grazing depth ($Z_{g\text{-}max}$, m)	13.0	5.8–20.0	—	
Physical				
Light extinction coefficient (K_p, m^{-1})	0.30	0.15–0.46	0.26–0.30	14
Lake depth (Z_m, m)	25.0	12.0–39.0	10.8–37.0	14
Maximum oxygen penetration (Z_o, m)	20.0	6.8–23.2	9.3–14.3	15

Note: All values indicated as percentages were implemented as probabilities.

*1, Leavitt and Carpenter (1990*b*); 2, Bianchi and Findlay (1991); 3, Sun et al. (1993); 4, Steenbergen et al. (1994); 5, Welschmeyer and Lorenzen (1985*b*); 6, Carpenter et al. (1986); 7, Head and Harris (1992); 8, Wetzel (1983); 9, Sommer (1988); 10, Butler and Dam (1994); 11, Reynolds (1984); 12, Welschmeyer and Lorenzen (1985*a*); 13, Pollingher (1988); 14, Cleugh and Hauser (1971); 15, Stefan et al. (1995).

posited in the sediments. Postdepositional losses (e.g., Hurley and Amstrong 1990, 1991) were not considered.

Rate constants for pigment loss processes were obtained from a comprehensive review of published mesocosm and whole-lake experiments and were usually characteristic of the carotenoid β-carotene (Table 1; see Leavitt 1993 and references therein). β-Carotene is a chemically stable, ubiquitous carotenoid (Goodwin 1980) used in paleolimnology to estimate past changes in total algal abundance (reviewed in Sanger 1988). Physical parameters included lake depth (Z_m), oxic zone depth (Z_o), photic depth (Z_p), and light extinction coefficient (K_p). Values for these parameters were based on deep (depth >5 m), unproductive lakes of the Experimental Lakes Area (ELA), Ontario (Cleugh and Hauser 1971). Unlike many locales, there are long-term historical data available for ELA, and the site has been the subject of recent investigations of pigment biogeochemistry (Leavitt and Findlay 1994; Leavitt et al. 1999).

As with all models, the model that we constructed represents a compromise between realism, explanatory power, and tractability. Consequently, this model includes all known loss processes but simplifies the physical characteristics of lakes. For example, simulated lakes exhibited exponential attenuation of light and hypolimnetic anoxia but not thermal or hydrodynamic stratification. Because physical stratification (i.e., turbulence, temperature) mainly regulates sinking and degradation rates, and because these rates were already controlled by unique parameters (see below), the model was viewed as a reasonable compromise. Similarly, although sediment focusing and resuspension also affect the settling of particulate matter (e.g., Crusius and Anderson 1995; Weyhenmeyer et al. 1997), these factors control the inputs to the suspended pigment pool rather than degradative processes and were not considered further.

Finally, this model did not allow for feedback among planktonic components (cf. Sarnelle 1999) or for seasonal changes in the relative magnitude of control parameters (e.g., light, herbivory, oxygen stratification). Seasonal patterns of pigment sedimentation could be produced by allowing relevant control parameters (e.g., light, oxygen, grazing, sinking velocity) to vary during each simulated year. However, because our principal goal was to understand and quantify complex factor interactions, most simulations were conducted by assuming steady-state, summerlike conditions.

In the model, each algal cell was characterized by depth (Z_i), degree of pigment degradation (percent), grazing status (grazed, ungrazed), and mortality status (live, dead). Computer simulations were initialized by assigning random values for each of these parameters to a given initial number of phytoplankton (Table 1). The values for these parameters were recorded in a matrix with a maximum size of 1000 algal cells. A standard run began after 100 days of simulation (to achieve steady state and minimize the effect of initial conditions) and was monitored for a further 630 days. The minimum time step of the model was 1 day. Model output included algal population size, pigment deposition rates (calculated from total number of cells sedimented per day and the percent pigment degradation per cell sedimented, where total pigment was given as 0.225 pg·cell^{-1} (Goodwin 1980)), vertical distribution of phytoplankton, and origin of sinking particles. The depth of each algal cell was calculated each day according to algal or fecal sinking rates (V_f, V_a) after applying all loss and recruitment processes.

Phytoplankton population growth was modelled as a probabilistic process. Low rates of phytoplankton recruitment, characteristic of unproductive systems, were used (Table 1). Each day, new algae were introduced at random depths within the photic zone. In most simulations, we assumed that populations were not vertically stratified. Initial cell recruitment (K_r) and death (K_d) were each set to a given rate and implemented as a probability (Table 1). Phytoplankton were recruited to the epilimnion only if matrix fullness was

© 1999 NRC Canada

Table 2. Effect of subroutine implementation sequence on predicted pigment deposition.

Sequence of model	Matrix size (cells)	Run length (years)	Pigment deposition ($pg \cdot day^{-1} \pm SD$)
R–G–P–O–S*	1000	2	1.767±0.010
G–P–O–S–R	1000	2	1.837±0.014
R–P–O–S–G	1000	2	1.914±0.013
R–G–S–P–O	1000	2	2.198±0.013
R–S–G–P–O	1000	2	2.200±0.012
S–R–G–P–O	1000	2	2.047±0.014
R–G–P–O–S	2000	2	2.021±0.009
R–G–P–O–S	1000	1	1.770±0.014

Note: P, photooxidation, O, oxidation, G, grazing, S, sedimentation, R, algal recruitment, SD, standard deviation of 75 simulations.
*Standard sequence.

less than 75% and the randomly assigned position in the matrix was empty. The finite size of the matrix mimics density-dependent growth of the algal population as it approaches the carrying capacity of the environment imposed by nutrient limitation or by algal self-shading. Using this method of implementing algal recruitment assumes that an increase in algal density would be felt equally at all depths in the photic zone (i.e., there are not locally different rates of algal recruitment at different depths within the photic zone). It also implies that dead cells occupying the water column would interfere in the rate of population growth (i.e., possibly by shading or attenuation of nutrients).

Death rates (K_d) were implemented as the probability that an individual algal cell would die during a time step (Table 1). The death rate was a minimum estimate of mortality arising solely from algal senescence. Algal cells were also killed by grazing and by permanent descent into anoxic water. Algal cells were removed from the simulation when they had reached the lake bottom or when their pigments had completely degraded.

Herbivory by zooplankton was assumed to occur within a grazing zone of a given thickness. Both minimum ($Z_{g\text{-}min}$) and maximum ($Z_{g\text{-}max}$) depths of grazing were varied (Table 1). Live algal cells within the grazing zone were ingested at a rate (V_g) implemented as a daily probability of ingestion (Table 1). We assumed that ingestion of phytoplankton by herbivores resulted in algal death and a substantial percentage of pigment degradation (K_g) (Table 1) (Head and Harris 1992) but that coprophagy was negligible (Lorenzen and Welschmeyer 1983; Sarnelle 1999). Grazed cells were incorporated into rapidly sinking, coherent fecal material. Mean fecal pellet sinking rate (V_f) was about eightfold greater than that of ungrazed phytoplankton (V_a) (Table 1). Fecal sinking rates represent a compromise between diffuse egesta of *Daphnia* (e.g., Uehlinger and Bloesch 1987; Leavitt and Carpenter 1990*b*) and highly coherent fecal pellets from large copepods (Lorenzen and Welschmeyer 1983; Butler and Dam 1994). Algal sinking rates were derived from in situ estimates (e.g., Reynolds 1984). Photooxidation and oxidation of pigments within fecal pellets were assumed to be negligible.

Pigments from dead phytoplankton within the photic zone were subject to photooxidation. The photic zone was defined as that region of the water column with greater than 1% of surface irradiance. As a simplifying assumption, the daily receipt of light at the lake surface was set at a constant 60 einsteins·m^{-2}, although this parameter could be varied to incorporate seasonal cycles of irradiance. Photooxidation rates were determined by the light flux at the depth of each algal cell, calculated assuming exponential attenuation of light with depth (K_p). Daily pigment photodegradation for each cell (P_{deg}) was given as

$$P_{deg} = P_{tot}(1 - e^{(-K_{p\text{-}ox})I_z})$$

where P_{tot} is the current total pigments in the algal cell, $K_{p\text{-}ox}$ is the pigment photooxidation coefficient, and I_z is the light flux at depth z (Carpenter et al. 1988). Light attenuation was assumed not to vary with the algal cell population. This assumption is probably justified for the range of algal standing crops seen in the oligotrophic lakes of ELA, where light penetration is mainly controlled by the content of dissolved organic carbon (Schindler et al. 1996), but may lead to overestimation of photooxidative losses in eutrophic systems.

Pigments from dead phytoplankton below the photic zone, but within the oxic zone, were subject to oxidation by chemical or microbial processes (e.g., Hurley and Armstrong 1990; Leavitt and Carpenter 1990*b*). We assumed that pigments within live cells and dead cells in fecal material were protected from additional pigment oxidation (Goodwin 1980; Carpenter et al. 1986). Phytoplankton pigment content following oxidation (P_{new}) was calculated daily as

$$P_{new} = P_{tot} - e^{-K_o}$$

where K_o is the oxidation coefficient. Estimates of K_o used in this study were obtained from in situ experiments with phytoplankton and are characteristic of stable carotenoids such as alloxanthin and lutein (Leavitt and Carpenter 1990*b*). Similar values of K_o have been obtained from experiments with other algal, plant, and sedimentary material (Hurley and Armstrong 1990; Bianchi and Findlay 1991; Steenbergen et al. 1994).

Model behaviour

The sensitivity of model output to the sequence of events (recruitment and degradative processes), matrix size, and simulation length was tested by running simulations with all possible combinations of event order and comparing mean daily pigment sedimentation rates. Model output was sensitive to the sequence of events during the simulations (Table 2). As expected, estimated sedimentation rates were 5–20% lower if pigments were degraded before cell sinking occurred. Sedimentation rates increased 12% as matrix size doubled because a few simulations experienced fluctuations in the algal cell population that exceeded the lower population maximum of the smaller matrix size. Sedimentation rates were insensitive to the length of the simulation (1–2 years). Consequently, all subsequent analyses used a standard 2-year simulation with a maximum of 1000 algal cells and fixed event sequence: recruitment, grazing, photooxidation, oxidation, and sedimentation.

© 1999 NRC Canada

Model predictions

The importance of parameters in influencing model behaviour and simulated pigment deposition was described by quantifying two properties: (*i*) the amount of variance in pigment flux produced by varying the model parameters by a known amount (sensitivity) and (*ii*) the absolute magnitude of pigment losses attributed to a given process. We used Monte Carlo error analysis to quantify effects of variations in model parameters on pigment flux. We estimated the potential magnitude of loss attributed to a given process (e.g., grazing) by eliminating that process from the model and measuring the resulting increase in simulated pigment deposition (magnitude analysis). In addition, we examined the effects of plankton vertical position, herbivory, and lake infilling in detail.

Monte Carlo error analyses

A Monte Carlo error analysis is a numerical approximation of a sensitivity analysis: a procedure used to determine the effect of parameter variation on model predictions (Bartell et al. 1986). In the numerical approximation of this procedure, model parameters are selected randomly over a given range of variance. The model under study is then used to produce a set of predictions where the variance of these predictions is a function of both parameter variability and the stochastic aspects of the model. This variance can then be examined statistically to quantify the dependence of the model predictions on variation in individual parameters (Bartell et al. 1986).

We described the range of each input parameter by a normal distribution with a given coefficient of variation (CV) of the mean. Parameter means and ranges were obtained from published mesocosm experiments and lake surveys (Table 1). Parameter values for individual simulations were selected from the generated distributions using a stratified, random sampling design (Iman and Conover 1980). Once selected, parameters were randomized and combined to produce 75 sets of lake conditions. Each set of conditions was used to produce one run of the model.

As recommended by Gardner et al. (1981), a small CV of 2% was used to approximate the results of a standard analytical sensitivity analysis. This analysis was also repeated using the same means but a larger CV (20%) to examine the effects of a wider range of parameter variance on model performance (O'Neill et al. 1980; Bartell et al. 1988). A single fixed variance was used for all parameters because it approximates the observed range of variance of some well-described variables (e.g., K_{ox}, $K_{p\text{-}ox}$, V_g, V_a; Table 1) and because there was no a priori rationale for assuming other distributions for parameters whose variance was either too imprecisely known (e.g., K_g, $Z_{g\text{-}max}$, $Z_{g\text{-}min}$; cf. Sarnelle 1999) or was unique to specific lake districts (e.g., Z_m, Z_p).

The importance of each parameter in determining the variance of the model predictions was ranked using the relative partial sum of squares from a linear multiple regression relating pigment deposition to parameter values (Bartell et al. 1988). Data generated by sets of model parameters that were unfeasible (e.g., photic depth greater than lake depth) were rare and were eliminated from the data set prior to analysis. Signs of the estimates from multiple regression models (+, –) and response surface plots were used to determine whether individual parameters increased or reduced pigment deposition.

Predicted pigment sedimentation was significantly influenced ($P < 0.05$) by variation in factors related to algal growth, lake morphology, herbivore grazing, and chemical oxidation (Fig. 2). In particular, variations in algal cell sinking rate, lake depth, pigment loss during grazing (digestion), maximum depth of oxygen penetration, algal recruitment, and fecal pellet sinking rate were significant predictors of pigment sedimentation, although their relative importance varied between the 2 and 20% CV analyses. Multiple linear regressions with these parameters explained 92 and 85% of the variation, respectively.

Pigment deposition was positively correlated with phytoplankton recruitment ("production") and algal sinking velocity but was unaffected by initial population size, likely because simulations were at steady state before sedimentation was measured (Table 3; Fig. 2). Variation in pigment digestion was the most important determinant of sedimentation rates among variables related to herbivory (Fig. 2). Digestive losses significantly decreased pigment deposition (Table 3) and explained the greatest amount of variance in deposition (Fig. 2). In contrast, predicted pigment deposition was positively correlated with fecal sinking rate and the grazing rate of herbivores (Table 3).

The effect of variation in photooxidation on predicted pigment sedimentation was unexpectedly slight compared with results from mesocosm experiments (Carpenter et al. 1986; Leavitt and Carpenter 1990*b*). Variation in photooxidation rate was only the seventh most important determinant of variation in pigment sedimentation in the 20% CV analysis (Fig. 2). Although rapid photooxidation reduced pigment deposition, sedimentation was positively correlated with photic zone depth (Table 3). Neither factor had a significant influence on predicted pigment sedimentation in analyses with 2% CV (Fig. 2). In contrast with photooxidation, phytoplankton pigment deposition was significantly altered by variation in chemical or microbial oxidation (Fig. 2). Pigment deposition was significantly ($P < 0.05$) and inversely correlated with the maximum depth of oxygen penetration but was unrelated to precise rate of oxidation over the range of variation tested.

Predicted pigment deposition declined with increased lake depth (Fig. 2; Table 3). Lake depth and algal recruitment also interacted to regulate pigment sedimentation (Fig. 3). Pigment deposition increased with phytoplankton recruitment (Table 3) but declined as lakes became deeper. The interaction was additive, with maximum deposition rates in shallow lakes with high algal production (Fig. 3).

Magnitude analysis

The Monte Carlo error analysis determines the sensitivity of the model predictions to parameter variation. However, the analysis does not imply that the greatest amount of pigment loss is due to the most sensitive parameter. We conducted a simple test to determine which processes (oxidation, bleaching, or grazing) were responsible for the largest amount of pigment loss in simulations.

The parameter values for the oxidation coefficient (K_o), the photooxidation coefficient ($K_{p\text{-}ox}$), and digestion by herbivores (K_g) were separately set to zero for three sets of

© 1999 NRC Canada

Fig. 2. Relative influence of variation in parameters on predicted pigment deposition (measured by relative partial sum of squares from Monte Carlo error analyses where CV = 2% of the mean (open bars) and CV = 20% of the mean (solid bars)). Where variation in model parameters significantly influenced predicted rates of pigment deposition ($P < 0.05$), bars extend beyond the vertical dashed lines (95% confidence limits).

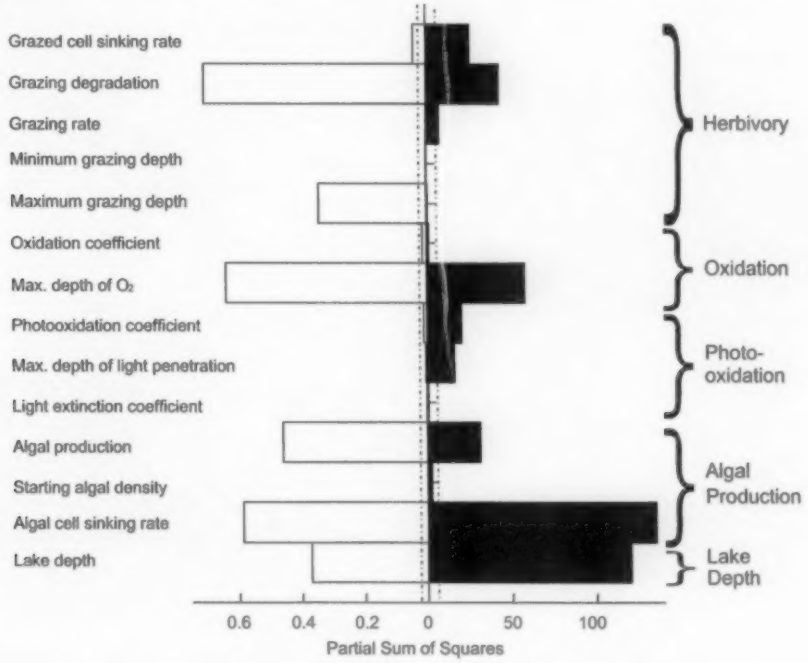


Table 3. Estimates from multiple regression analysis of the effect of variation in model parameters on predicted pigment deposition rate (slope) and significance of these estimates (P value).

Parameter	Slope estimate	P
Biological		
Oxidation coefficient (K_o)	–5.097	0.4045
Photooxidation coefficient ($K_{p\text{-}ox}$)	–22.34	0.0016
Grazing degradation (K_g)	–2.857	0.0001
Grazing rate (V_g)	1.265	0.0511
Feces sinking rate (V_f)	0.318	0.0004
Phytoplankton sinking rate (V_a)	5.638	0.0001
Phytoplankton recruitment (K_r)	2.856	0.0001
Initial algal abundance (P_i)	–0.001	0.2649
Minimum grazing depth ($Z_{g\text{-}min}$)	–737.4	0.8331
Physical		
Light extinction coefficient (K_p)	–1.427	0.5476
Lake depth (Z_m)	–0.122	0.0001
Photic zone depth (Z_p)	0.141	0.0048
Maximum oxygen penetration (Z_o)	–0.121	0.0001

Note: CV is 20% of the parameter mean.

model runs in which all other parameters were sampled from a distribution with a CV of 20%. In each set, the mean rate of pigment deposition for 150 model runs was calculated and compared with a control set of model runs in which all loss parameters were sampled from normal distributions. Mean pigment deposition was compared among sets of simulations using ANOVA.

The magnitude of the effects of herbivory, photooxidation, and oxidation on pigment sedimentation was significantly different (ANOVA; $F_{3,436} = 16.10$, $P < 0.0001$). Duncan's multiple range test showed that the mean (± SD) amount of pigment sedimented in the two sets of simulations in which herbivory (5.28 ± 3.62 pg·day^{-1}) or photooxidation (4.37 ± 2.83 pg·day^{-1}) was set to zero was significantly greater than in the control simulation in which all three loss variables were sampled from a 20% CV normal distribution (2.95 ± 2.05 pg·day^{-1}). The largest loss of pigments was due to herbivore grazing. Pigment deposition rates were 2.5 times greater in the absence of digestion by herbivores. Photooxidation was the next largest loss process and pigment sedimentation rates were twofold greater in the absence of photooxidation. When pigments were protected from chemical oxidation, the mean sedimentation was not significantly different from the control. (3.38 ± 2.04 pg·day^{-1}).

Effects of phytoplankton depth

We examined the hypothesis that pigments from metalimnetic phytoplankton were selectively transported to deep waters (Baines and Pace 1994) by monitoring the origin and fate of algal pigments in the simulated water column. Initial algal position in the water column was randomized and mean daily deposition rate estimated for

© 1999 NRC Canada

Fig. 3. Predicted pigment deposition as a function of lake depth and algal recruitment for parameter values randomly selected from a normal distribution with 20% CV with means from Table 1.

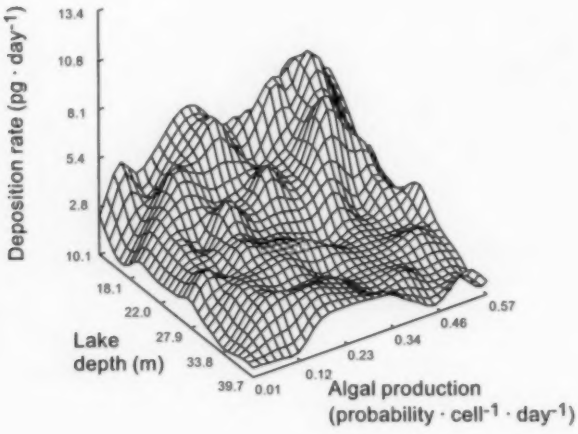
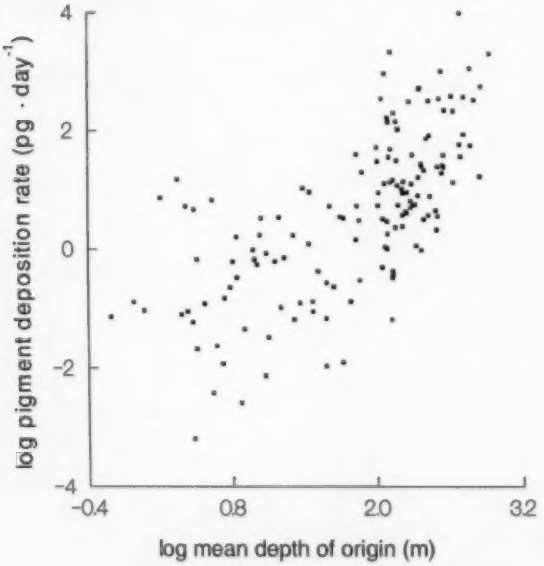
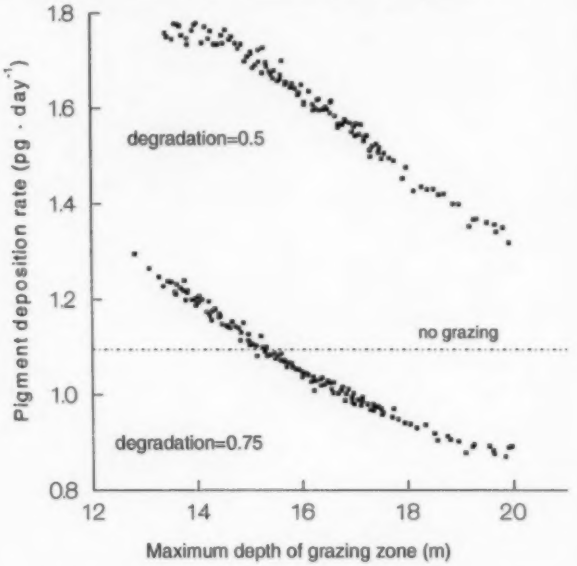


Fig. 4. Predicted pigment deposition versus mean phytoplankton position in the water column for simulations with constant herbivory and pigment oxidation and all other parameters randomly selected from a normal distribution with 20% CV with means from Table 1.



150 simulations with rates of herbivory, photooxidation, and chemical oxidation held constant with values given in Table 1. All other variables were sampled from a distribution with 20% CV (Table 1). Pearson correlation coefficients of log-transformed data were used to test the correlation between mean initial phytoplankton depth and mean daily deposition rate. In addition, mean pigment sedimentation rates were compared using t tests for standard simulations in which

Fig. 5. Predicted pigment deposition versus the depth of herbivores with a fixed 12-m-thick grazing zone. Herbivores degrade either 50 or 75% of all ingested pigments. All other parameters are randomly selected from a normal distribution with 20% CV with means from Table 1. The horizontal dashed line indicates deposition in the absence of grazing (ingestion = 0).



phytoplankton were restricted to either the epilimnion (mean cell depth = 4.5 m) or the metalimnion (mean cell depth = 10.8 m).

The model predicted that pigment deposition was biased towards phytoplankton that originated deep in the water column (Fig. 4). Variation in mean depth of origin explained 47% ($P < 0.001$) of the variation in pigment deposition in simulations with constant rates of herbivory, photooxidation, and chemical oxidation. Similarly, deposition of pigments restricted to a metalimnetic origin (6.34 pg pigment·day^{-1}) was significantly different from and threefold greater than that of epilimnetic pigments (2.04 pg pigment·day^{-1}).

Effects of herbivory

We also examined the hypothesis that grazing by zooplankton increases the rate of pigment deposition (Welschmeyer and Lorenzen 1985*b*; Carpenter et al. 1988) despite substantial pigment digestion during gut passage (Head and Harris 1992). Pigment deposition in the absence of grazers ($V_g = 0$) was estimated from 75 simulations under otherwise standard conditions (Table 1). When present, herbivores were assumed to destroy either 50 or 75% of ingested pigments, values within the natural range of digestive losses (Conover et al. 1986; Head and Harris 1992).

The influence of zooplankton vertical distribution on pigment deposition was also examined by allowing the depth of a 12-m-thick grazing zone to vary within the oxic zone (Z_o = 20 m). We assumed that herbivores could not exploit the deeper anoxic water. Effects of herbivores on pigment deposition were estimated by calculating pigment sedimentation during 75 simulations in which digestion rate was fixed but

© 1999 NRC Canada

Table 4. Limnological parameters for Big Lake, British Columbia, summer 1994.

Characteristic	Value/description
Latitude	51°40.1′
Longitude	121°27.0′
Elevation (m above sea level)	1030
Area (ha)	110
Mean depth (m)	3.2
Maximum depth (m)	9.1
Original depth (m)	19.4
Oxygen penetration (m)	7.0
Photic zone (m)	5.0
pH	9.0
Dissolved solids ($g \cdot L^{-1}$)	0.37
Conductivity ($S \cdot cm^{-1}$)	880
Fish	Stocked trout
Zooplankton	Unknown

Note: Data from Bennett et al. (2000) and unpublished.

grazing zone depth varied. All other input variables were held constant.

Pigment deposition was greater in the presence of herbivores than in their absence, but only if digestive losses were low or if herbivores occurred high in the water column (Fig. 5). For example, herbivores that degraded 50% of ingested pigments consistently increased pigment deposition relative to ungrazed reference simulations. In contrast, when digestive losses were increased to 75%, herbivores only increased deposition when they occurred within the upper 15 m of the water column. In general, pigment deposition declined with the depth of herbivores, although the exact relationship depended on the degree of pigment digestion.

Effects of lake infilling

We examined the effects of lake infilling on the predicted rate of pigment deposition to determine how regulatory processes might vary during Holocene lake ontogeny. Standard paleoecological analyses suggest that lakes become more productive through time, at least partly due to biogeochemical changes associated with basin infilling (e.g., Wetzel 1983). However, this view does not consider the possibility that processes regulating fossil deposition, and hence inferred production, are likely to change with lake morphometry (Davis and Ford 1982). To address this issue, we compared pigment flux from model simulations with actual observations of historical changes in fossil pigments during the Holocene. In addition, we conducted Monte Carlo analyses for four sets of model parameters representative of past lake states during the Holocene to simulate how the relative importance of control parameters might have changed during lake infilling. Parameter values were based on Big Lake, British Columbia, Canada (Table 4), a 9.1-m-deep lake for which the basic limnology and paleoecology are known (Bennett et al. 2000).

Big Lake has accumulated 10 m of sediment (~50% of original basin depth) over the Holocene era. As a simplifying assumption, we considered that present lake level elevation has not varied substantially and that the original 19.4-m-deep basin has simply become shallower through time. Actual lake depths may vary in a more complicated pattern related to historical changes in climate and water balance (Bennett et al. 2000). The parameters of photic depth (5.0 m), depth of oxygen penetration (7.0 m), and the width of the grazing zone (0.001–7.001 m) reflect the current conditions of this lake.

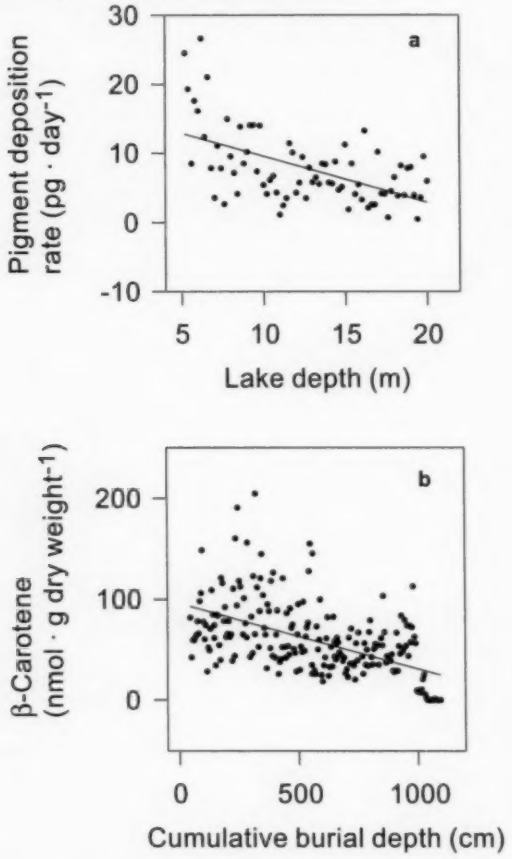
Model output was compared with changes in concentrations of fossil β-carotene from Big Lake sediment to determine whether fossil-inferred changes in postglacial production might arise as artifacts of basin infilling (e.g., Davis and Ford 1982; Leavitt 1993). A set of 75 simulations were completed in which all model parameters except algal production (where production was constant) were selected from a randomized stratified sampling of a normal distribution about the means in Table 1 with 20% CV. Maximum lake depth was initially 19.4 m and was allowed to vary over the range of lake depths inferred from paleoecological analyses of Big Lake. The relationship between changes in lake depth and the simulated pigment flux was compared with that observed from changes in fossil pigment concentration (nanomoles of pigment per gram of organic matter) from an actual sediment core over the same range of lake depths. To make this comparison for data in different units, a generalized linear model (GLM) procedure in SAS was used to compare the linear slopes of pigments versus lake depth following standardization of both data sets (converted to Z distributions with $\mu = 0$ and $\sigma^2 = 1$; Steel and Torrie 1980).

Both the simulation model and the sediment core analyses indicated that pigment deposition increased as lake depth declined (Fig. 6). Variation in lake depth explained similar amounts of variation in pigments for regressions based on simulation ($r^2 = 0.28$, $F_{1,74} = 26.36$, $P < 0.001$) and paleoecological data ($r^2 = 0.30$, $F_{1,212} = 89.9$, $P < 0.001$). For the standardized data, there was no significant difference between the slopes of lake depth versus rate of pigment deposition predicted by the simulation model and the relationship between the concentration of β-carotene and estimated lake depth from the sediment core ($F_{1,284} = 0.00$, $P = 0.9889$), regardless of whether data were log transformed or not. However, given the small fraction of variance explained, agreement between the slopes should be interpreted as indicating that there is qualitative rather than quantitative agreement between the model predictions and the sediment core. The similarity between fossil and model regressions, given that simulations did not allow algal production to vary through time, suggests that paleoecological inferences of past lake production and ontogeny may be biased by changes in lake morphology.

A Monte Carlo error analysis was used to determine whether sensitivity to variation in factors related to pigment deposition changed with a change in lake depth. Four sets of model simulations (225 runs each) were conducted for the lake depth values of 19.4, 16.1, 13.0, and 9.7 m, which spanned the range of depth experienced by Big Lake during the Holocene. The analysis indicated that predicted pigment deposition was more sensitive to variation in algal recruitment in shallow systems (Fig. 7). Overall, the importance of variation in recruitment increased at the expense of both algal sinking rate and digestive losses to grazers. The importance of variation in other model parameters did not change

© 1999 NRC Canada

Fig. 6. (*a*) Predicted pigment deposition during lake infilling from a simulation model with parameter values selected from a normal distribution with 20% CV about means given in Table 1, unless otherwise indicated in the text. (*b*) β-Carotene concentration versus cumulative burial depth in a sediment core from Big Lake, British Columbia. The solid lines are relationships estimated by linear regression.



significantly with lake infilling (Fig. 7). These patterns suggest that paleoecological analyses in shallow lakes may be more sensitive to historical changes in lake production than similar studies of deeper basins.

Discussion

Pigment sedimentation has been used to estimate a variety of aquatic processes including temporal changes in algal production and community structure (Millie et al. 1993), vertical distribution of algae (Hurley and Watras 1992), plankton sedimentation (Poister et al. 1999), herbivory (Welschmeyer and Lorenzen 1985*a*; Carpenter et al. 1988), and ecosystem efficiency (Baines and Pace 1994). Yet despite numerous empirical studies describing the patterns of pigment sedimentation (reviewed in Leavitt 1993) and experimentation to identify possible regulatory factors (Hurley and Armstrong 1990, 1991; Leavitt and Carpenter 1990*a*, 1990*b*; Bianchi and Findlay 1991; Steenbergen et al. 1994), there have been few attempts to synthesize these studies or to develop a conceptual framework for studying pigment sedimentation.

Our individual-based model was constructed to improve the understanding of pigment sedimentation, examine interactions among regulatory mechanisms that were not amenable to direct manipulation, and generate hypotheses to help reconcile disparate observations from previous empirical and experimental studies. In the construction of any model, simplifications are necessary in order to provide insights into otherwise intractable systems. In this model, we assumed that thermal structure and turbulent mixing regime were adequately represented by parameters that control the rates of pigment degradation and cell sinking, respectively, and that these factors did not require additional unique parameters (e.g., temperature-dependent decomposition, depth-stratified sinking rates). This version of the model did not allow for feedback among planktonic components (cf. Sarnelle 1999) or for seasonal changes in the relative magnitude of control parameters (e.g., light, herbivory, oxygen stratification). Variation associated with short-term biological feedbacks within the plankton (e.g., size-refugia from grazing, nutrient recycling, ecological stoichiometry) is less likely to be important in controlling interannual patterns of pigment sedimentation than are fundamental state changes in lake structure (e.g., eutrophication, fish introduction, acidification), as shown clearly from mass balance studies, whole-lake experiments, and comparisons of long-term limnological records and annual sedimentary profiles (Carpenter et al. 1988; Leavitt et al. 1989, 1994, 1999; Leavitt and Findlay 1994). As our goal was to describe interannual patterns of deposition, and because most algal growth and pigment deposition occurs during the summer stratified period in our model lake district (ELA) (Leavitt and Findlay 1994; Leavitt et al. 1994, 1999), reduced annual variance associated with eliminating seasonal variation (e.g., winter) was also viewed as a reasonable simplification.

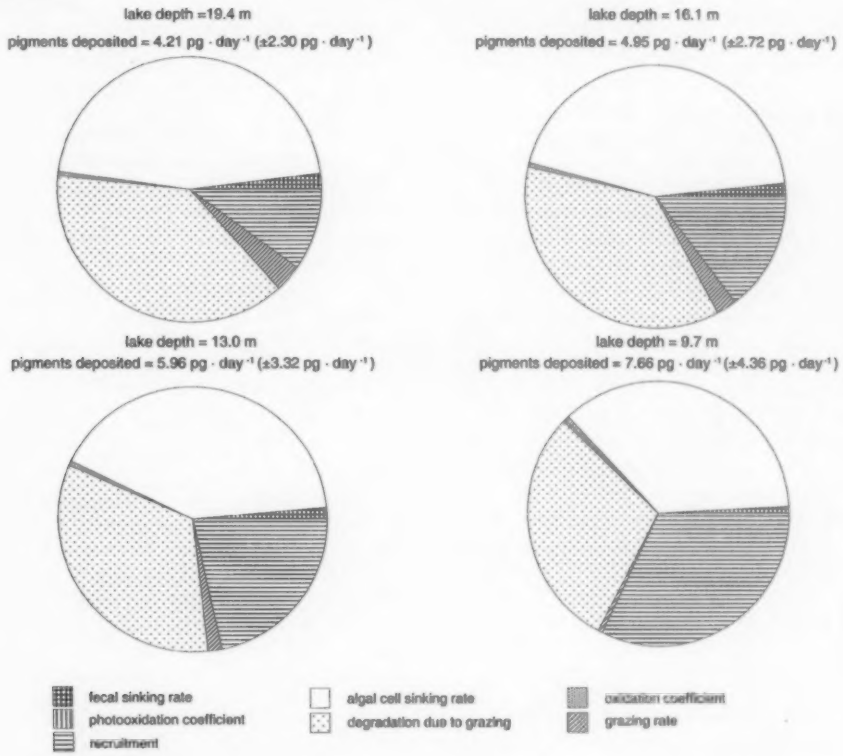
The model predicts that pigment deposition is regulated by complex interactions among primary production, lake morphology, pigment oxidation, vertical structure of plankton communities, and grazing by herbivores. Pigment flux was correlated with primary production, particularly when the physical properties of lakes were relatively constant (depth, oxygen penetration) or when pigments originated from deepwater populations. Although herbivores degraded pigments, grazing increased vertical pigment flux when digestive losses were less than pigment oxidation during phytoplankton sedimentation. In addition, simulations suggested that reconstructions of past algal production may be predictably biased because of systematic changes in regulation of pigment deposition arising from basin infilling.

Algal production

Pigment sedimentation was significantly and positively related to variance in algal production in our simulation model (Fig. 2; Table 3). These results are consistent with empirical observations that phytoplankton standing crop and pigment deposition are correlated over a range of production (e.g., Gorham et al. 1974; Guilizzoni et al. 1983; Leavitt and Findlay 1994; Leavitt et al. 1999), despite rapid pigment degradation within the water column due to oxidation (e.g.,

© 1999 NRC Canada

Fig. 7. Relative influence of variation in parameters on predicted pigment deposition (ranked by relative partial sum of squares from Monte Carlo error analyses where CV = 20% of the mean) for Big Lake, British Columbia, under four scenarios of decreasing lake depth during the Holocene.


lake depth =19.4 m
pigments deposited = 4.21 pg · day⁻¹ (±2.30 pg · day⁻¹)
lake depth = 16.1 m
pigments deposited = 4.95 pg · day⁻¹ (±2.72 pg · day⁻¹)
lake depth = 13.0 m
pigments deposited = 5.96 pg · day⁻¹ (±3.32 pg · day⁻¹)
lake depth = 9.7 m
pigments deposited = 7.66 pg · day⁻¹ (±4.36 pg · day⁻¹)
fecal sinking rate
photooxidation coefficient
recruitment
algal cell sinking rate
degradation due to grazing
oxidation coefficient
grazing rate

Leavitt and Carpenter 1990*a*; Hurley and Armstrong 1991), photooxidation (e.g., Welschmeyer and Lorenzen 1985*b*; Carpenter et al. 1986), and grazing (e.g., Welschmeyer and Lorenzen 1985*b*; Head and Harris 1992).

The relationship between algal production and lake depth (Fig. 3) leads to the prediction that correlations between phytoplankton production and pigment deposition should be strongest when lake morphometry and chemical stratification are relatively constant. Consistent with this hypothesis, Adams and Prentki (1986) demonstrated an exceptional correspondence ($r^2 = 0.97$, $P < 0.01$) between past lake production and sedimentary pigment concentrations in Lake Mead, whereas Carpenter et al. (1986) were unable to demonstrate any significant correlation in oligomesotrophic lakes that had high variation in the depth of oxygen penetration but similar algal abundance. Similarly, Vinebrooke et al. (1998) documented relatively weak correlations between water column and fossil pigments ($r = 0.21–0.33$, $P < 0.001$) in a survey of western Canadian lakes. Although Guilizzoni et al. (1983) demonstrated that production and deposition remain correlated despite variable lake morphometry ($r^2 = 0.80$, $P < 0.001$), this result may reflect the low variation in light and oxygen penetration among these eutrophic lakes (Guilizzoni et al. 1982). As well, eutrophic conditions normally promote pigment preservation (Swain 1985; Sanger 1988). Regardless, our analyses suggest that the potential dependence of pigment sedimentation rates on lake morphometry should be taken into account when comparisons of productivity are inferred from sediment traps and bottom samples for lakes of different depths.

Pigment oxidation

In general, the model predicted that the magnitude of chemical oxidation of pigments in these simulated lakes is set by the length of exposure to oxygen rather than the precise rate of loss. Predicted rates of pigment deposition were positively related to cell sinking rate and inversely related to the thickness of the oxic zone but were unrelated to the rate of oxidation (Fig. 2; Table 3).

In situ incubations (Leavitt and Carpenter 1990*a*; Bianchi and Findlay 1991), mass balance budgets (Leavitt and Carpenter 1990*b*), and comparisons of pigments in traps and sediments (Hurley and Armstrong 1990, 1991; Steenbergen et al. 1994) also demonstrate that pigment losses increase with exposure to oxygen. As well, accumulation of organic carbon in marine sediments has been similarly related to oxygen exposure (Hartnett et al. 1998). Consistent with predictions from empirical studies (Swain 1985; Sanger 1988; Leavitt 1993), our model predicts that factors that reduce the length of exposure (sinking rate, transit distance, fecal packaging) will increase pigment deposition.

The prediction that the pigment sedimentation is regulated mainly by the balance between production and the length of time exposed to oxidative conditions is robust to most spe-

© 1999 NRC Canada

cific assumptions of stratification. In this model, lake simulations included exponential attenuation of irradiance and hypolimnetic anoxia but not thermal or hydrodynamic stratification. Hypolimnetic waters were considered anoxic and pigments were protected from further degradation, consistent with both in situ experiments (Leavitt and Carpenter 1990*b*; Bianchi and Findlay 1991; Sun et al. 1993) and empirical studies (Hurley and Armstrong 1990, 1991; Steenbergen et al. 1994). Consequently, changes in deepwater pigment sedimentation or degradation rates resulting from vertical differences in water temperature or turbulence have little effect on pigment deposition and could not influence model predictions.

The amount of pigment lost to photooxidation was second only to that resulting from herbivores. These results are similar to those from mass balance budget, which show that bleaching accounts for substantial pigment loss (Welschmeyer and Lorenzen 1985*a*, 1985*b*; Carpenter et al. 1986, 1988; Leavitt and Carpenter 1990*b*). However, because these mass balance studies did not incorporate digestive losses of pigments due to zooplankton, it is difficult to evaluate the prediction that grazing accounts for more pigment loss than does photooxidation. In addition, pigment sedimentation in our simulations was not sensitive to variation in photooxidation coefficients ($K_{p\text{-}ox}$) (Fig. 2) even though the estimated natural range of values was well approximated by our model (Table 1). Therefore, the simulation model suggests that photooxidation is a relatively blunt control of pigment deposition and that inferences of algal community composition will not be greatly biased by selective photooxidation arising from pigment-specific differences in $K_{p\text{-}ox}$.

The prediction that photooxidation exerts only coarse controls on pigment sedimentation suggests that artifacts in estimation of total photooxidative losses from microcosm-derived $K_{p\text{-}ox}$ values do not greatly bias mass balance studies of pigment sedimentation (e.g., Carpenter et al. 1986, 1988). Normally, $K_{p\text{-}ox}$ is estimated by killing algae and allowing them to bleach in suspended containers (e.g., Leavitt and Carpenter 1990*a*). Values of $K_{p\text{-}ox}$ are obtained from regressions of pigment concentration against photon flux. Total pigment photooxidation is estimated by applying these $K_{p\text{-}ox}$ values to vertical profiles of light and pigment concentration (Welschmeyer and Lorenzen 1985*b*; Carpenter et al. 1986; Leavitt and Carpenter 1990*b*). However, this procedure may overestimate the importance of photooxidation because it assumes that phytoplankton are dead prior to exposure to light, both in original determinations of $K_{p\text{-}ox}$ and in applications of the decay coefficients to field data. Instead, pigments are continuously produced in live phytoplankton and are protected from net photooxidation (Goodwin 1980) in all but extreme cases (e.g., Paerl et al. 1984). As well, a significant proportion of algal mortality arises from sedimentation into aphotic or anoxic waters, conditions that do not lead to photooxidation of pigments (St. Amand and Carpenter 1993). The observation that total photooxidation accounted for a large amount of pigment loss over a range of $K_{p\text{-}ox}$ values suggests that while mass balance budgets tend to overestimate $K_{p\text{-}ox}$, this bias may have little impact on estimates of total photooxidative losses.

Phytoplankton depth

As demonstrated by empirical studies (Brown et al. 1984; Hurley and Garrison 1993; Baines and Pace 1994), mean phytoplankton depth was an important determinant of total pigment deposition (Fig. 2). In our model, pigments from metalimnetic algae (Z_{avg} = 10.8 m) were three times more likely to be deposited at lake bottom than were those originating from epilimnetic populations (Z_{avg} = 4.5 m). Baines and Pace (1994) have used regression models to show that metalimnetic algal abundance is a better predictor of vertical pigment flux than is epilimnetic biomass. Similarly, paleolimnological studies show that sedimentary deposits contain high proportions of undegraded pigments from deepwater populations of chrysophytes (Leavitt et al. 1994) or phototrophic bacteria (Brown et al. 1984; Hurley and Garrison 1993). Our model suggests that such consistent underestimation of epilimnetic pigment flux arises because of pigment oxidation during sinking (Fig. 2) and further suggests that most mass balance and paleolimnological studies will underestimate total algal production when epilimnetic phytoplankton account for a high proportion of total algal abundance.

Herbivory

Field and laboratory experiments show that the magnitude of actual digestive losses depends on several factors including pigment lability, nutritional history of the grazers, feeding rate or food abundance, and the species of phytoplankton and herbivores (reviewed in Leavitt 1993). In our standard simulations, degradation of pigments to colourless derivatives was always significant. Digestion by herbivores was the largest source of pigment loss in the magnitude analyses but has not previously been incorporated into mass balance budgets of pigment flux.

The effect of herbivores on pigment deposition depended on the magnitude of digestive losses, sinking rate of feces, and the position of both phytoplankton and herbivores within the water column. As suggested by others (Welschmeyer and Lorenzen 1985*b*; Carpenter et al. 1986, 1988; Leavitt et al. 1989), the model predicts that incorporation of pigments into fecal material may bypass oxidative losses in the water column. Grazing should increase the rate of pigment deposition in circumstances where the sum of digestive losses is less than the sum of oxidative losses incurred when ungrazed phytoplankton settle to the lake bottom. Herbivores increased pigment deposition when digestive losses were low or when herbivores occurred high in the water column (Fig. 5). However, lakes that are dominated by those herbivores that produce less coherent fecal matter (e.g., *Daphnia*) will likely suffer much greater photooxidation and oxidative losses of pigments in sedimenting material. In addition, our model predicts that epilimnetic herbivores increase pigment deposition more than metalimnetic grazers. In lakes where migratory zooplankton are common, the exact impact of zooplankton on pigment flux should also depend on the relative amount of time that herbivores spend in shallow and deep waters. This hypothesis might be tested by comparisons of pigment flux in lakes with contrasting migratory zooplankton but similar algal production and vertical distribution, such as can occur following food web manipulations (e.g., Dini and Carpenter 1988).

The model suggests that changes in community structure, as reflected in variation in algae-specific (K_o, $K_{p\text{-}ox}$, K_g, K_d,

© 1999 NRC Canada

K_r, V_a) and zooplankton-specific parameters (V_g, V_f), can substantially alter rates of pigment deposition. This prediction agrees with experimental (Carpenter et al. 1988) and paleolimnological analyses (Kitchell and Carpenter 1987; Leavitt et al. 1989). The model also predicts that rapidly sinking algae will be more likely to be well preserved in sediment traps and at lake bottom, consistent with the empirical findings of Poister et al. (1999). Similarly, herbivores with more coherent fecal pellets should increase the rate of pigment sedimentation more than those with diffuse egesta (e.g., Kitchell and Carpenter 1987). Finally, the mean position of both phytoplankton and herbivores in the water column will also determine the rate of pigment deposition. Together, these relationships open up the possibility of inferring shifts in community structure through pigment analysis (e.g., Carpenter et al. 1988; Claustre 1994). However, further use of in situ experimentation and modelling will be required to fully explore the role of species-level interactions in regulating pigment flux in lakes.

Lake infilling

Simulations with parameters specific to Big Lake (Table 4) predicted that a decrease in lake depth, with no concomitant change in algal production parameters, caused an increase in the amount of pigment incorporated into the sediments (Fig. 6*a*). This prediction was borne out in direct analyses of sedimentary β-carotene concentrations, which increased steadily during the Holocene as sediments accumulated. These comparisons suggest that inferred increases in production during lake ontogeny may partly arise from artifacts associated with long-term changes in lake morphometry rather than solely from increased production. Although speculative, this conclusion reaffirms the need for further studies on the variability of pigment sedimentation before Holocene reconstructions can be reliably interpreted.

Future directions

We believe that future studies in paleolimnology should include modelling approaches. Ultimately, our goal is to generate a family of models to allow neo- and paleoecologists to use highly resolved sedimentary deposits to quantify past lake production and food web structure in units equivalent to modern measurements. However, further research is required before such predictive models can be used confidently.

Subsequent model versions could be modified to examine the roll of seasonal variation in regulating the formation of paleoecological records and the influence that season-specific climatic change might have on the pigment sedimentation processes. Realistic patterns of seasonal variation in regulatory factors (e.g., light, grazing, algal recruitment, oxygen) can be added by letting parameter means vary appropriately during seasonal simulations. State changes can be produced in the current model by implementing large changes (e.g., to correspond to whole-lake manipulations) or gradual shifts (e.g., to reflect natural interannual changes in community structure) in individual algal and invertebrate parameters (e.g., grazing rate, production rate). However, improved estimates are required for virtually all model parameters (Table 1), as in most cases there were insufficient data to adequately quantify their natural range and because known parameters were derived from studies with different taxa, design, and habitats. Given the high sensitivity of model output to the magnitude of parameter variance (Fig. 2), we argue that further experimentation be conducted prior to application of our results to lakes of widely varying physical and biological properties. Finally, specific predictions of these models must be validated with appropriate experiments and field studies (cf. Sarnelle 1999).

Ultimately, we anticipate that these models will provide a valuable theoretical basis for biogeochemical and paleoecological studies of pigments and other labile fossils (e.g., fatty acids, chitinous remains, organic contaminants). Paleolimnology frequently provides the sole source of information on past effects of humans and climatic change on lakes (Binford et al. 1983; Sanger 1988), but this science currently lacks a strong theoretical basis for describing processes that form fossil records.

Acknowledgements

This research was supported by the Natural Sciences and Engineering Research Council of Canada and by the University of Regina.

References

Adams, M.S., and Prentki, R.T. 1986. Sedimentary pigments as an index of the trophic status of Lake Mead. Hydrobiologia, **143**: 71–77.

Aksnes, D.L., and Wassman, P. 1993. Modelling the significance of zooplankton grazing for export production. Limnol. Oceanogr. **38**: 978–985.

Baines, S.B., and Pace, M.L. 1994. Relationships between suspended particulate matter and sinking flux along a trophic gradient and implications for the fate of planktonic primary production. Can. J. Fish. Aquat. Sci. **51**: 25–36.

Bartell, S.M., Breck, J.E., Gardner, R.H., and Brenkert, A.L. 1986. Individual parameter perturbation and error analysis of fish bioenergetics models. Can. J. Fish. Aquat. Sci. **43**: 160–168.

Bartell, S.M., Brenkert, A.L., and Carpenter, S.R. 1988. Parameter uncertainty and the behavior of a size-dependent plankton model. Ecol. Model. **40**: 85–95.

Bennett, J., Cumming, B.F., Leavitt, P.R., Chiu, M., Smol, J.P., and Szeicz, J. 2000. A multiproxy paleolimnological perspective on climatic changes from Big Lake, Cariboo Plateau, British Columbia, Canada. Quat. Res. In press.

Bianchi, T.S., and Findlay, S. 1991. Decomposition of Hudson estuary macrophytes: photosynthetic pigment transformations and decay constants. Estuaries, **14**: 67–73.

Bianchi, T.S., Findlay, S., and Dawson, R. 1993. Organic matter sources in the water column and sediments of the Hudson River estuary: the use of plant pigments as tracers. Estuarine Coastal Shelf Sci. **36**: 359–376.

Binford, M.W., Deevey, E.S., and Crisman, T.L. 1983. Paleolimnology: an historical perspective on lacustrine ecosystems. Annu. Rev. Ecol. Syst. **14**: 255–286.

Brown, S.R., McIntosh, H.J., and Smol, J.P. 1984. Recent paleolimnology of a meromictic lake. Fossil pigments of photosynthetic bacteria. Verh. Int. Ver. Limnol. **22**: 1357–1360.

Butler, M., and Dam, H. 1994. Production rates and characteristics of fecal pellets of the copepod *Acartia tonsa* under simulated phytoplankton bloom conditions: implications for vertical fluxes. Mar. Ecol. Prog. Ser. **114**: 81–91.

© 1999 NRC Canada

Carpenter, S.R., Elser, M.M., and Elser, J.J. 1986. Chlorophyll production, sedimentation and degradation: implications for paleolimnology. Limnol. Oceanogr. **31**: 112–124.

Carpenter, S.R., Leavitt, P.R.,. Elser, J.J., and Elser, M.M. 1988. Chlorophyll budgets: response to food web manipulations. Biogeochemistry, **6**: 79–90.

Claustre, H. 1994. The trophic status of various oceanic provinces as revealed by phytoplankton pigment signatures. Limnol. Oceanogr. **39**: 1206–1210.

Cleugh, T.R., and Hauser, B.W. 1971. Results of the initial survey of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. **28**: 129–137.

Conover, R.J., Durvasula, R., Roy, S., and Wang, R. 1986. Probable loss of chlorophyll-derived pigments during passage through the gut of zooplankton and some of the consequences. Limnol. Oceanogr. **31**: 878–887.

Crusius, J., and Anderson, R.F. 1995. Sediment focusing in six small lakes inferred from radionuclide profiles. J. Paleolimnol. **13**: 143–155.

Davis, M.B., and Ford, M.S. 1982. Sediment focussing in Mirror Lake, New Hampshire. Limnol. Oceanogr. **27**: 137–150.

DeAngelis, D.L., and Gross, L.J. 1992. Individual-based models and approaches in ecology: populations, communities and ecosystems. Chapman and Hall, New York.

Dini, M.L., and Carpenter, S.R. 1988. Variability in *Daphnia* behavior following fish community manipulations. J. Plankton Res. **10**: 621–635.

Gardner, R.H., O'Neill, R.V., Mankin, J.B., and Carney, J.H. 1981. A comparison of sensitivity analysis and error analysis based on a stream ecosystem model. Ecol. Model. **12**: 173–190.

Goodwin, T.W. 1980. The biochemistry of carotenoids. Vol. 1. Plants. Chapman and Hall, New York.

Gorham, E., Lund, J.W.G., Sanger, J.E., and Dean, W.E., Jr. 1974. Some relationships between algal standing crop, water chemistry, and sediment chemistry in the English Lakes. Limnol. Oceanogr. **19**: 601–617.

Guilizzoni, P., Bonomi, G., Galanti, G., and Ruggiu, D. 1982. Basic trophic status and recent development of some Italian lakes as revealed by plant pigments and other chemical components in sediment cores. Mem. Ist. Ital. Idrobiol. Dott Marco Marchi, **40**: 79–98.

Guilizzoni, P., Bonomi, G., Galanti, G., and Ruggiu, D. 1983. Relationship between sedimentary pigments and primary production: evidence from core analyses of twelve Italian lakes. Hydrobiologia, **103**: 103–106.

Hartnett, H.E., Keill, R.G., Hedges, J.I., and Devol, A.H. 1998. Influence of oxygen exposure time on organic carbon preservation in continental margin sediments. Nature (Lond.), **391**: 572–575.

Head, E.J.H., and Harris, L.R. 1992. Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. Mar. Ecol. Prog. Ser. **86**: 229–238.

Hurley, J.P., and Armstrong, D.E. 1990. Fluxes and transformations of aquatic pigments in Lake Mendota, Wisconsin. Limnol. Oceanogr. **35**: 384–398.

Hurley, J.P., and Armstrong, D.E. 1991. Pigment preservation in lake sediments: a comparison of sedimentary environments in Trout Lake, Wisconsin, USA. Can. J. Fish. Aquat. Sci. **48**: 472–486.

Hurley, J.P., and Garrison, P.J. 1993. Composition and sedimentation of aquatic pigments associated with deep plankton in lakes. Can. J. Fish. Aquat. Sci. **50**: 2713–2722.

Hurley, J.P., and Watras, C.J. 1992. Identification of bacteriochlorophylls in lakes via reverse-phase HPLC. Limnol. Oceanogr. **36**: 307–315.

Huston, M., DeAngelis, D., and Post, W. 1988. New computer models unify ecological theory. BioScience, **38**: 682–691.

Iman, R.L., and Conover, W.J. 1980. Small sample sensitivity analysis techniques for computer models, with an application to risk assessment. Commun. Stat. **A9**: 1749–1842.

Kitchell, J.F., and Carpenter, S.R. 1987. Piscivores, planktivores, fossils and phorbims. *In* Predation: direct and indirect impacts on aquatic communities. *Edited by* W.C. Kerfoot and A. Sih. University Press of New England, Hanover, N.H. pp. 136–146.

Leavitt, P.R. 1993. A review of factors that regulate carotenoid and chlorophyll deposition and fossil pigment abundance. J. Paleolimnol. **9**: 109–127.

Leavitt, P.R., and Carpenter, S.R. 1990*a*. Regulation of pigment sedimentation by photo-oxidation and herbivore grazing. Can. J. Fish. Aquat. Sci. **47**: 1166–1176.

Leavitt, P.R., and Carpenter, S.R. 1990*b*. Aphotic pigment degradation in the hypolimnion: implications for sedimentation studies and paleolimnology. Limnol. Oceanogr. **35**: 520–534.

Leavitt, P.R., and Findlay, D.L. 1994. Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. **51**: 2286–2299.

Leavitt, P.R., Carpenter, S.R., and Kitchell, J.F. 1989. Whole-lake experiments: the annual record of fossil pigments and zooplankton. Limnol. Oceanogr. **34**: 700–717.

Leavitt, P.R., Sanford, P.R., Carpenter, S.R., and Kitchell, J.F. 1994. An annual fossil record of production, planktivory and piscivory during whole-lake manipulations. J. Paleolimnol. **11**: 133–149.

Leavitt, P.R., Findlay, D.L., Hall, R.I., and Smol, J.P. 1999. Algal responses to dissolved organic matter loss and pH decline during whole-lake acidification: evidence from paleolimnology. Limnol. Oceanogr. **44**: 757–773.

Lorenzen, C.J., and Welschmeyer, N.A. 1983. The in situ sinking rates of herbivore fecal pellets. J. Plankton Res. **5**: 929–933.

Millie, D.F., Paerl, H.W., and Hurley, J.P. 1993. Microalgal pigment assessments using high-performance liquid chromatography: a synopsis of organismal and ecological applications. Can. J. Fish. Aquat. Sci. **50**: 2513–2527.

O'Neill, R.V., Gardner, R.H., and Mankin, J.B. 1980. Analysis of parameter error in a nonlinear model. Ecol. Model. **49**: 667–685.

Paerl, H.W., Tucker, J., and Bland, P.T. 1984. Carotenoid enhancement and its role in maintaining blue-green algal *Microcystis aeruginosa* surface blooms. Limnol. Oceanogr. **28**: 847–857.

Poister, D., Armstrong, D.E., and Hurley, J.P. 1999. Influences of grazing on temporal patterns of algal pigments in suspended and sedimenting algae in a north temperate lake. Can. J. Fish. Aquat. Sci. **56**: 60–69.

Pollingher, U. 1988. Freshwater armored dinoflagellates: growth, reproduction strategies and population dynamics. *In* Growth and reproductive strategies of freshwater phytoplankton. *Edited by* C.D. Sandgren. Cambridge University Press, Cambridge, U.K. pp. 134–174.

Repeta, D.J., and Gagosian, R.B. 1984. Transformation reactions and recycling of carotenoids and chlorins in the Peru upwelling region (15°S, 75°W). Geochim. Cosmochim. Acta, **48**: 1265–1277.

Reynolds, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge, U.K.

Sanger, J.E. 1988. Fossil pigments in paleoecology and paleolimnology. Palaeogeogr. Palaeoclimatol. Palaeoecol. **62**: 343–359.

Sarnelle, O. 1999. Zooplankton effects on vertical particulate flux: testable models and experimental results. Limnol. Oceanogr. **44**: 357–370.

© 1999 NRC Canada

Schindler, D.W., Bailey, S.E., Parker, B.R., Beaty, K.G., Cruikshank, D.R., Fee, E.J., Schindler, E.U., and Stainton, M.P. 1996. The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. Limnol. Oceanogr. **41**: 1004–1017.

Sinninghe Damste, J.S., and Koopmans, M.P. 1997. The fate of carotenoids in sediments: an overview. Pure Appl. Chem. **69**: 2067–2074.

Sommer, U. 1988. Growth and survival strategies of planktonic diatoms. *In* Growth and reproductive strategies of freshwater phytoplankton. *Edited by* C.D. Sandgren. Cambridge University Press, Cambridge, U.K. pp. 227–260.

St. Amand, A.L., and Carpenter, S.R. 1993. Metalimnetic phytoplankton dynamics. *In* The trophic cascade in lakes. *Edited by* S.R. Carpenter and J.F. Kitchell. Cambridge University Press, Cambridge, U.K. pp. 210–224.

Steel, R.G.D., and Torrie, J.H. 1980. Principles and procedures of statistics. McGraw-Hill Inc., New York.

Steenbergen, C.L.M., Korthals, H.J., and Dobrynin, E.J. 1994. Algal and bacterial pigments in non-laminated lacustrine sediment: studies of their sedimentation, degradation and stratigraphy. FEMS Microbiol. Ecol. **13**: 335–352.

Stefan, H., Fang, X., Wright, D., Eaton, J., and McCormick, J.H. 1995. Simulation of dissolved oxygen profiles in a transparent dimictic lake. Limnol. Oceanogr. **40**: 105–118.

Sun, M.-Y., Lee, C., and Aller, R.C. 1993. Laboratory studies of oxic and anoxic degradation of Chl *a* in Long Island Sound sediments. Geochim. Cosmochim. Acta, **57**: 147–157.

Swain, E.B. 1985. Measurement and interpretation of sedimentary pigments. Freshwater Biol. **15**: 53–76.

Uehlinger, U., and Bloesch, J. 1987. The influence of crustacean zooplankton on the size structure of algal biomass and suspended and settling seston (biomanipulation in limnocorrals II.). Int. Rev. Gesamten Hydrobiol. **72**: 473–486.

Vinebrooke, R.D., Hall, R.I., Leavitt, P.R., and Cumming, B.F. 1998. Fossil pigments as indicators of phototrophic response to salinity and climate change in lakes of western Canada. Can. J. Fish. Aquat. Sci. **55**: 668–681.

Welschmeyer, N.A., and Lorenzen, C.J. 1985*a*. Role of herbivory in controlling phytoplankton abundance: annual pigment budget for a temperate marine fjord. Mar. Biol. (Berl.), **90**: 75–86.

Welschmeyer, N., and Lorenzen, C. 1985*b*. Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific Gyres. Limnol. Oceanogr. **30**: 1–21.

Wetzel, R.G. 1983. Limnology. Saunders, Toronto, Ont.

Weyhenmeyer, G.A., Hakanson, L., and Meili, M. 1997. A validated model for daily variations in the flux, origin, and distribution of settling particles within lakes. Limnol. Oceanogr. **42**: 1517–1529.

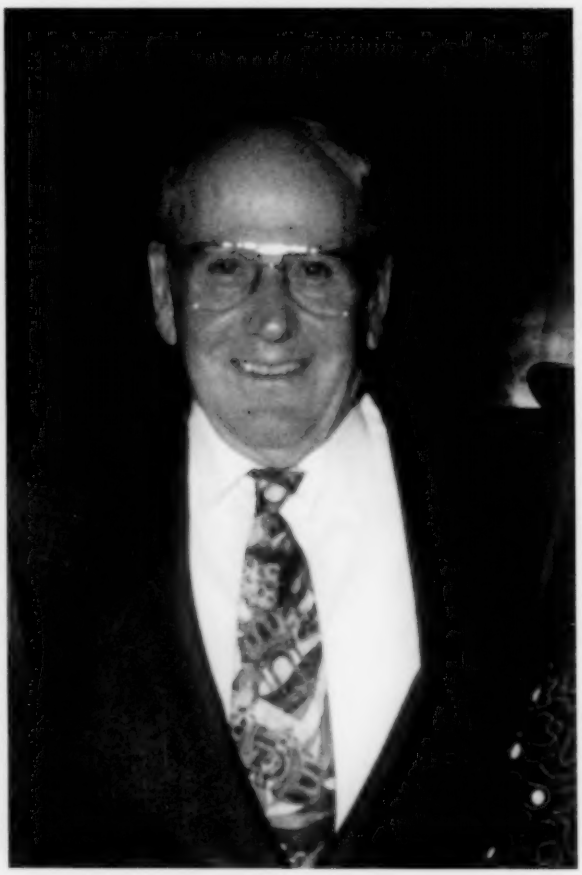
Yamazaki, H., Osborn, T.R., and Squires, K.D. 1991. Direct numerical simulation of planktonic contact in turbulent flow. J. Plankton Res. **13**: 629–643.

© 1999 NRC Canada

OBITUARY / NÉCROLOGIE

In Memoriam — Terrance Henry Butler (1923–1998)

Terrance (Terry) Henry Butler, a recognised world authority in the field of crustacean biology and crustacean fisheries, passed away on March 10, 1998, in Nanaimo, British Columbia, Canada. He was 74 years old.



Terry was born in Nelson, British Columbia, where his father worked as an engineer on the Canadian Pacific ferries. He received his elementary and secondary education in the interior of British Columbia (B.C.) and, prior to World War II, entered the engineering department of Victoria College in Victoria, B.C. In 1942, he joined the Canadian army, enlisting in the Engineer Corps. He transferred to the Canadian Airborne Regiment, with whom he served overseas, and was stationed in the Netherlands by the end of the War. He was discharged from the army in January 1946.

Upon returning to Canada, he enrolled in the University of British Columbia, where he completed a BA degree in Honours Biology in 1949. He received an MA degree from the same University in 1953; the subject of his thesis was a study of Dungeness crab biology and fisheries.

During the summer of 1948, Terry had been hired as a summer student at the Pacific Biological Station in Nanaimo, B.C., where he served as a groundfish technician conducting biological sampling of commercial groundfish catches. Thus began an association with the Biological Station that lasted for the remainder of his working career. In May 1949, he accepted a permanent position at the Pacific Biological Station as a research scientist in charge of the Crab and Shrimp Investigation. He continued in this capacity, also serving for a period as head of the Shellfish Investigation, until he retired from the Pacific Biological Station in March 1984.

During his working career, Terry undertook research studies on a wide range of subjects that included basic biology, population dynamics and stock assessment of some British Columbia crustacean species, exploratory fishing for harvestable stocks, and improvements in fishing methods. He became a

Nécrologie — Terrance Henry Butler (1923–1998)

Terrance (Terry) Henry Butler, autorité reconnue mondialement en matière de biologie et de pêche des crustacés, est décédé le 10 mars 1998, à Nanaimo, en Colombie-Britannique, au Canada. Il était âgé de 74 ans.

Terry est né à Nelson, en Colombie-Britannique, ville où son père était ingénieur sur les transbordeurs du Canadien Pacifique. Il a fait son cours primaire et secondaire dans l'intérieur de la Colombie-Britannique et, avant la Seconde Guerre mondiale, il étudiait à la faculté d'ingénierie du Victoria College, à Victoria. En 1942, il a rejoint les rangs de l'Armée canadienne, dans le corps de génie. Il a été transféré au Régiment aéroporté du Canada, dans lequel il a servi outre-mer; lorsque la guerre a fini, il était en poste aux Pays-Bas. Il a été démobilisé en janvier 1946.

À son retour au Canada, il s'est inscrit à l'Université de la Colombie-Britannique, où, en 1949, il a reçu son baccalauréat spécialisé en biologie. En 1953, il a obtenu une maîtrise de la même université; sa thèse portait sur l'étude de la biologie et de la pêche du crabe dormeur.

En 1948, alors étudiant, Terry a obtenu un emploi d'été en tant que technicien des poissons de fond à la Station biologique du Pacifique, à Nanaimo, en Colombie-Britannique. Il effectuait des échantillonnages biologiques des prises commerciales de poisson de fond. Ainsi débuta son association avec la Station biologique, qui a duré le reste de sa carrière. En mai 1949, Terry a accepté un poste permanent de chercheur scientifique à la Station : il était responsable des recherches sur les crabes et les crevettes. Il a conservé ce poste jusqu'à sa retraite en mars 1984, et pendant un certain temps, il a été chef de la recherche sur les invertébrés.

Durant sa carrière, Terry a entrepris des recherches sur une grande variété de sujets, comme la biologie fondamentale, la dynamique des populations et l'évaluation des stocks de certaines espèces de crustacés de la Colombie-Britannique, la pêche exploratoire des stocks exploitables et l'amélioration des mé-

© 1999 NRC Canada

recognised authority on crustacean fisheries of the West Coast of North America. As a result of his work, several crustacean fisheries were started in British Columbia. Throughout his working career, Terry maintained a close working relationship with the industry and was frequently consulted by the industry for his advice.

Terry published widely in the field of crustacean biology and fisheries, having over 55 publications including his "Shrimps of the Pacific Coast of Canada," which has remained a classic reference work. He received the Queen's Silver Jubilee Medal in 1977 in recognition of his career.

Terry also had an active interest in crustacean fisheries in developing countries. From January 1957 to November 1958, he served with the Food and Agriculture Organisation of the United Nations in Indonesia, assisting in the development and management of local shrimp fisheries. In 1988, he served with the Canadian Executive Services Organisation to assist in the development of a southern king crab fishery in Chile.

After his retirement in 1984, Terry continued to work several days a week at the Pacific Biological Station, even when his health was failing, to continue with his studies and publications on British Columbia crustaceans. At the time of his passing, he was writing a major work, "The Crab Fisheries of British Columbia." Terry was highly respected for his research accomplishments and was an inspiration to younger scientists at the Pacific Biological Station and elsewhere. He was a kind and patient man who always had time to talk with younger staff members, to encourage them in their work, and to give them the benefit of his long years of experience.

Terry had many outside interests. He was keenly interested in plants, shrubs, and trees and always claimed that he was a frustrated botanist. Wherever he travelled, he was very interested in the local fauna and how it compared to that in British Columbia. He had a great appreciation of classical music and he was an enthusiastic and much respected golfer and a regular member of a Saturday morning golfing group from the Pacific Biological Station.

Terry married D. Joan Abel in 1947; they celebrated their golden wedding anniversary in 1997. Together, they had six children, nine grandchildren, and a great granddaughter.

Terry was a warm-hearted, kind, and generous companion. Those who knew him appreciated his understanding, his guidance, and his example and these qualities served to inspire his associates and to assist them in their research and in their daily lives.

Terry will be sorely missed by his family and by all who knew him.

thodes de pêche. Il est ainsi devenu un éminent spécialiste de la pêche des crustacés de la côte ouest nord-américaine; son travail a permis d'ailleurs l'ouverture de plusieurs pêches en Colombie-Britannique. Durant l'ensemble de sa carrière, Terry a conservé une étroite relation de travail avec l'industrie, dont les membres l'ont fréquemment consulté pour obtenir des conseils.

Terry a publié beaucoup de documents sur la biologie et la pêche des crustacés; parmi ses 55 publications, « Shrimps of the Pacific Coast of Canada » est un ouvrage de référence classique. En 1977, il a reçu la Médaille commémorative du règne de la Reine pour l'ensemble de sa carrière.

Terry s'intéressait aussi de près à l'ouverture de pêches de crustacés dans les pays en développement. De janvier 1957 à novembre 1958, il a travaillé à l'Organisation des Nations Unies pour l'alimentation et l'agriculture, et est allé en Indonésie pour aider à construire et à gérer la pêche locale de la crevette. En 1988, il a aidé le Service d'assistance canadien aux organismes à développer la pêche du crabe royal antarctique au Chili.

Après sa retraite en 1984, Terry a continué de travailler plusieurs jours par semaine à la Station biologique du Pacifique, même lorsque sa santé se détériorait, afin de continuer ses recherches et ses publications sur les crustacés de la Colombie-Britannique. À sa mort, il rédigeait une œuvre majeure sur la pêche du crabe en Colombie-Britannique intitulée : « The Crab Fisheries of British Columbia ». Terry était profondément respecté pour ses découvertes scientifiques et était une source d'inspiration pour les jeunes chercheurs de la Station biologique et d'ailleurs. Aimable et patient, il avait toujours le temps de discuter avec les jeunes membres de l'équipe, de les encourager dans leurs recherches et de les faire bénéficier de sa longue expérience.

Terry avait beaucoup d'autres centres d'intérêt. C'était un mordu des plantes, des arbustes et des arbres; il a toujours affirmé qu'il était un botaniste frustré. Partout où il voyageait, il était fortement intéressé par la faune locale, et la comparait à celle de la Colombie-Britannique. C'était un grand amateur de musique classique; un golfeur passionné et très respecté, il faisait partie du groupe de golf du samedi matin de la Station biologique du Pacifique.

Terry a épousé D. Joan Abel en 1947; ils ont célébré leurs noces d'or en 1997. Ils ont eu ensemble six enfants, neuf petits-enfants et une arrière petite-fille.

Terry était une personne chaleureuse, gentille et généreuse. Ceux qui le connaissaient appréciaient sa compréhension et ses conseils, et le prenaient en exemple; ces qualités ont inspiré ses compagnons de travail dans leurs vies professionnelles et personnelles.

La famille de Terry et tous ceux qui le connaissaient se souviendront de lui avec émotion.

Partial Publication List for T.H. Butler / Liste partielle des publications de T.H. Butler

Butler, T.H. 1949. The status of the pink shrimp (Prawn), *Pandalus borealis* Kroyer, in the commercial shrimp fishery of English Bay British Columbia. B.A. thesis, University of British Columbia.

Butler, T.H. 1950. The commercial shrimps of British Columbia. Fish. Res. Board Can., Pacific Coast Station Prog. Rep. **83**: 30–34.

Butler, T.H. 1950. Two records of shrimps from English Bay, B.C. Can. Field-Nat. **64**(5).

Butler, T.H. 1951. The 1949 and 1950 tagging experiments in the Graham Is. Crab fishery. Fish. Res. Board Can., Pacific Coast Station Prog. Rep. **89**: 88–87.

Butler, T.H. 1953. The appearance of a new commercial shrimp in a newly developed shrimp fishery. Fish. Res. Board Can., Pacific Coast Station Prog. Rep. **94**: 30–31.

Butler, T.H. 1953. A shrimp survey by the "Investigator No. 1," April, 1953. Fish. Res. Board Can., Nanaimo Biol. Station Circular 55.

Butler, T.H. 1953. The life of the commercial crab. Western Fisheries, 1953.

© 1999 NRC Canada

Butler, T.H. 1954. Food of the commercial crab in the Queen Charlotte Islands Region. Fish. Res. Board Can., Pacific Coast Station Prog. Rep. **99**: 3–5.

Butler, T.H. 1955. Re-discovery of the parasitic cirripede, *Mycetomorpha vancouverensis* Potts, in British Columbia waters. J. Parasitol. **41**(3): 321.

Butler, T.H. 1956. The distribution and abundance of early postlarval stages of British Columbia commercial crab. Fish. Res. Board Can., Pacific Coast Station Prog. Rep. 107: 22–23.

Butler, T.H. 1956. A first British Columbia record of a cragonid shrimp. Can.Field-Nat. **70**(30): 142.

Butler, T.H. 1957. The tagging of the commercial crab in the Queen Charlotte Islands region. Fish. Res. Board Can., Pacific Coast Station Prog. Rep. **109**: 16–19.

Butler, T.H. 1959. Results of shrimp trawling by "Investigator No.1," June 1959. Fish. Res. Board Can., Nanaimo Biol. Station Circular 55.

Butler, T.H. 1960. Maturity and breeding of the Pacific edible crab, *Cancer magister* Dana. J. Fish. Res. Board Can. **17**(5): 641–646.

Butler, T.H. 1961. Records of decapod Crustacea from British Columbia. Can. J. Zool. **39**: 59–62.

Butler, T.H. 1961. Growth and age determination of the Pacific edible crab *Cancer magister* Dana. J. Fish. Res. Board Can. **18**: 873–891.

Butler, T.H. 1963. An improved prawn trap. Fish. Res. Board Can., Nanaimo Biol. Station Circular 67.

Butler, T.H. 1963. The prawn *Pandalus platyceros* Brandt 1851. FAO Fish. Rep. 57(4).

Butler, T.H. 1964. Records of shrimps (Order Decapoda) from British Columbia. J. Fish. Res. Board Can. **21**: 419–421.

Butler, T.H. 1964. Redescription of the parasitic isopod *Holophryxus alaskenis* Richardson and a note on it synonymy. J. Fish. Res. Board Can. **21**: 971–976.

Butler, T.H. 1964. Growth, reproduction, and distribution of pandalid shrimps in British Columbia. J. Fish. Res. Board Can. **21**: 1403–1452.

Butler, T.H. 1967. A bibliography of the Dungeness crab, *Cancer magister* Dana. Fish. Res. Board Can. Tech. Rep. 1.

Butler, T.H. 1967. Shrimp exploration and fishing in the Gulf of Alaska and Bering Sea. Fish. Res. Board Can. Tech. Rep. 18.

Butler, T.H. 1968. The shrimp fishery of British Columbia. FAO Fish. Rep. **57**(2): 521–526.

Butler, T.H. 1969. Catch and effort statistics of the Canadian shrimp fishery on the Pacific Coast in 1967. Fish. Res. Board Can. MS Rep. 1031.

Butler, T.H. 1970. Synopsis of biological data on the prawn *Pandalus platyceros* Brandt, 1851. FAO Fish. Rep. **57**(4): 1289–1315.

Butler, T.H. 1971. A review of the biology of the pink shrimp, *Pandalus borealis* Kroyer 1838. Can. Fish. Rep. **17**: 17–24.

Butler, T.H. 1971. *Eualus berkeleyorum* n.sp., and records of other caridean shrimps (Order Decapoda) from British Columbia. J. Fish. Res. Board Can. **28**: 1615–1620.

Butler, T.H. 1980. Catch and effort statistics of the Canadian shrimp fishery on the Pacific Coast in 1979. Can. Data Rep. Fish. Aquat. Sci. **224**.

Butler, T.H. 1980. Shrimps of the Pacific coast of Canada. Can. Bull. Fish. Aquat. Sci. **202**.

Butler, T.H. 1981. Dungeness crab: a primer. Western Fish. **103**(3): 26–27.

Butler, T.H., and Dubokovic, G.V. 1955. Shrimp prospecting in the offshore region of the British Columbia coast, June to August, 1953. Fish. Res. Board Can., Nanaimo Biol. Station Circular 39.

Butler, T.H., and Dubokobic, G.V. 1955. Shrimp and prawn prospecting on the British Columbia coast, June to December, 1954. Fish. Res. Board Can., Nanaimo Biol. Station Gen. Ser. Circ. 35.

Butler, T.H., and Hankin, D.G. 1992. Comment on mortality rates of Dungeness crabs *Cancer magister*. Can. J. Fish. Aquat. Sci. **49**: 1518–1521.

Butler, T.H., and Legare, H.E.J. 1954. Shrimp prospecting in regions of the British Columbia coast, November 1953 to March 1954. Fish. Res. Board Can., Gen. Ser. Circ. 31.

Butler, T.H., Lindsay, J.G., and Chic, C.B. 1975. Prawn trap exploration British Columbia Central coast, November 1974 to February 1975. Fish. Res. Board Can. MS Rep. 1357.

Butler, T.H., and Smith, M.S. 1968. Shrimp sampling and temperature data obtained during exploratory fishing off British Columbia, 1966 and 1967. Fish. Res. Board Can., Tech. Rep. 61.

Butler, T.H., and Stocker, M. 1990. Surplus production model analysis of crab (*Cancer magister* Dana) fisheries of British Columbia, Canada. Fish. Res. **9**: 231–254.

Butler, T.H., Yates, A.N., and Wood, C.C. 1973. *G.B. Reed* shrimp cruise 73-S-1, May 7–23. Fish. Res. Board Can. MS Rep. 1255.

Butler, T.H., Yates, A.N., and Miller, D.C. 1974. *G.B. Reed* shrimp cruise in Queen Charlotte Sound, April 1974. Fish. Res. Board Can., Nanaimo Biol. Station Gen. Ser. Circ. 98.

Allen, J.A., and Butler, T.H. 1994. The Caridea (Decapoda) collected by the Mid-Pacific Mountains Expedition, 1968. Pac. Sci. **48**(4): 410–445.

Boutillier, J.A., Butler, T.H., et al. 1998. Assessment of the "Area A" crab (*Cancer magister*) fishery in British Columbia. Can. Stock Assessment Secr. Res. Doc. 98/86.

Boutillier, J.A., Carmichael, J.R., and Butler, T.H. 1978. Shrimp population survey, west coast of Vancouver Island, May 1978. Can. Fish. Mar. Serv. Data Rep. 100(1).

Boutillier, J.A., Yates, A.N., and Butler, T.H. 1976. *G.B. Reed* shrimp cruise 76-S-1, May 3–19, 1976. Can. Fish. Mar. Serv. Data Rep. 13.

Boutillier, J.A., Yates, A.N., and Butler, T.H. 1977. *G.B. Reed* shrimp cruise 77-5-1, May 3–14. Can. Fish. Mar. Serv. Data Rep. 37.

Hankin, D.G., and Butler, T.H. 1997. Does intense fishing on males impair success of female Dungeness crabs? Can. J. Fish. Aquat. Sci. **54**(3): 655–669.

Jamieson, G.S., Robinson, C.K., and Butler, T.H. 1986. King and Tanner Crabs in northern British Columbia mainland inlets. Can. MS Rep. Fish. Aquat. Sci. 1880.

Ketchen, K.S., Bourne, N., and Butler, T.H. 1983. History and present status of fisheries for marine fishes and invertebrates in the Strait of Georgia, British Columbia. Can. J. Fish. Aquat. Sci. **40**: 1095–1119.

Margolis, L., and Butler, T.H. 1954. An unusual and heavy infection of a prawn, *Pandalus borealis* Kroyer, by a nematode, *Contracaecum* sp. J. Patasitol. **40**(6): 649–655.

Schrivener, J.C., and Butler, T.H. 1971. A bibliography of shrimps of the family Pandalidae, emphasizing economically important species of the genus *Pandalus*. Fish. Res. Board Can. Tech. Rep. 241.

Wicksten, M.K., and Butler, T.H. 1983. Description of *Eualus lineatus* new species, with a redescription of *Heptacarpus herdmani* (Walker), (Caridea: Hippolytidae). Proc. Biol. Soc. Washington, Washington, D.C., **96**(1): 1–6.

© 1999 NRC Canada

ASSIGNMENT OF COPYRIGHT

Ms. No. _____

To: NATIONAL RESEARCH COUNCIL OF CANADA ("NRC") Research Press

From: _____

_____

_____

_____

(name and address of author signing this form)

This refers to the manuscript entitled: _____

_____

written by myself and the following additional author(s): _____

_____

for publication in: _____

1. I declare that the list of authors fairly conforms to academic conventions about who should be listed. I declare that the manuscript, or its equivalent, has not been submitted for publication elsewhere. If it is accepted for publication by the National Research Council of Canada, I will ensure that it, or its equivalent, is not submitted for publication elsewhere.

2. I declare that I am (check ONE):

☐ (a)	the sole owner of the copyright relating to the manuscript,	and → I **assign** to NRC the entire copyright.
☐ (b)	a part owner of the copyright, in conjunction with interests held by co-authors or their employers,	and → I **assign** to NRC my entire interest in the copyright, and I expect the co-authors to also sign a form like this one.
☐ (c)	an agent of my employer with authority to assign the copyright owned by the employer, who is: _____,	and → I **assign** to NRC, on behalf of my employer, my employer's entire interest in the copyright relating to the manuscript, and I expect the co-authors to also sign a form like this one.
☐ (d)	a public servant in Canada (federal, provincial or territorial) or the United Kingdom, so that the copyright is owned by Her Majesty,	and → I have obtained the approval of my employer, according to the terms of my employment, to grant NRC **non-exclusive permission** to publish the manuscript and administer the copyright.
☐ (e)	a public servant of the United States of America, and subject to the law that creates no U.S. copyright for the manuscript,	and → I **assign** to NRC, on behalf of my employer, my employer's entire interest in any copyright that may exist outside the U.S., and I expect the co-authors to sign a form like this one.

3. I understand that my submission of this document does not guarantee publication, and that publication will not occur until **all authors** have provided a copyright transfer. If the manuscript is not published within 2 years after I sign this, the assignment is revoked.

Signed at _____ on _____

(City and Province/State) (Date)

_____

(Signature)

[Standard NRC form; alterations not acceptable.]

Comments on Copyright Transfer
(with extracts from the *Copyright Act*, Revised Statutes of Canada 1985, chapter C-42, as amended)

The publisher is not permitted to publish the manuscript without permission or an assignment of ownership of copyright. For greatest flexibility, and to be prepared to deal with future uses of the published material, the publisher requires ownership.

The owner may assign the right, but that assignment must be in writing:

13.(4) The owner of the copyright in any work may assign the right, either wholly or partially, and either generally or subject to territorial limitations, and either for the whole term of the copyright or for any other part thereof, and may grant any interest in the right by licence, but no assignment or grant is valid unless it is in writing signed by the owner of the right in respect of which the assignment or grant is made, or by his duly authorized agent.

The owner may be the author, but in many cases, the owner is the author's employer:

13.(3) Where the author of a work was in the employment of some other person under a contract of service or apprenticeship and the work was made in the course of his employment by that person, the person by whom the author was employed shall, in the absence of any agreement to the contrary, be the first owner of the copyright, but where the work is an article or other contribution to a newspaper, magazine or similar periodical, there shall, in the absence of any agreement to the contrary, be deemed to be reserved to the author a right to restrain the publication of the work, otherwise than as part of a newspaper, magazine or similar periodical.

The only exception to taking ownership arises when the manuscript is covered by Crown copyright owned by a Canadian government.

12. Without prejudice to any rights or privileges of the Crown, where any work is, or has been, prepared or published by or under the direction or control of Her Majesty or any government department, the copyright in the work shall, subject to any agreement with the author, belong to Her Majesty and in that case shall continue for the remainder of the calendar year of the first publication of the work and for a period of fifty years following the end of that calendar year.

Governments, and in particular the federal government, do not have a simple mechanism for assigning ownership of this particular type of Crown asset. Therefore, when an author is a public servant so that there is Crown copyright in the manuscript, a simple permission to use is acceptable to the publisher, if it is granted by a person with appropriate authority.

CESSION DU DROIT D'AUTEUR

Document n° _____

Au : CONSEIL NATIONAL DE RECHERCHES DU CANADA («CNRC») - Presses scientifiques

De : _____

_____

_____

_____

(nom et adresse de l'auteur qui signe le présent formulaire)

La présente cession porte sur le document intitulé : _____

_____

rédigé par moi, en collaboration avec l'auteur/les auteurs suivant(s) : _____

_____

aux fins de publication dans : _____

1. Je déclare que la liste des auteurs ci-dessus respecte les pratiques usuelles de rédaction savante quant aux personnes dont le nom doit être cité. Je déclare que le document ou son équivalent n'a pas été soumis à quelque autre organisme aux fins de publication. Si le CNRC accepte de publier le document, je m'assurerai que celui-ci ou son équivalent n'est soumis à aucun autre organisme aux fins de publication sans en obtenir la permission préalable du CNRC.

2. Je déclare être (UN SEUL choix) :

☐	(a)	le seul titulaire de droit d'auteur afférent au document,	et → je **cède** le droit d'auteur intégral au CNRC.
☐	(b)	un titulaire partite du droit d'auteur, de concert avec des coauteurs ou leurs employeurs,	et → je **cède** ma part du droit d'auteur au CNRC et m'attends à ce que les coauteurs signent une entente comparable.
☐	(c)	un agent de mon employeur ayant l'autorité de céder le droit d'auteur appartenant à l'employeur, nommément : _____,	et → je **cède** au CNRC, au nom de mon employeur, la part entière du droit d'auteur afférent au document appartenant à mon employeur, et m'attends à ce que les coauteurs signent une entente comparable.
☐	(d)	un fonctionnaire du Canada (fédéral, provincial ou territorial) ou du Royaume-Uni, donc le droit d'auteur appartient à Sa Majesté,	et → j'ai obtenu de mon employeur, en vertu de mes conditions d'emploi, l'autorisation d'accorder au CNRC la **permission non exclusive** de publier le document et de gérer le droit d'auteur.
☐	(e)	un fonctionnaire des États-Unis d'Amérique, donc sujet à la loi qui n'associe aucun droit d'auteur américain au document,	et → je **cède** au CNRC, au nom de mon employeur, la part entière de quelque droit d'auteur en vigueur ailleurs qu'aux É.-U. appartenant à mon employeur, et m'attends à ce que les coauteurs signent une entente comparable.

3. Je comprends que la soumission du document ne garantit aucunement sa publication et que la publication ne pourra être réalisée tant que **tous les auteurs** n'auront pas signé une cession du droit d'auteur. Si le document n'est pas publié dans les deux années suivant ma signature des présentes, la cession sera automatiquement révoquée.

Signé à _____, le _____

(ville et province/état) (date)

_____

(signature)

[Formulaire normalisé du CNRC; aucune modification permise.]

Commentaires sur la cession du droit d'auteur
(accompagnés d'extraits de la *Loi sur le droit d'auteur*, Lois révisées du Canada 1985, chapitre C-42 modifié)

L'éditeur ne peut publier un document sans la permission ou une cession du droit d'auteur. Pour des raisons de souplesse et en vue de l'utilisation future du document publié, l'éditeur doit devenir titulaire du droit d'auteur.

Le titulaire peut céder le droit, mais cette cession doit être faite par écrit :

13.(4) Le titulaire du droit d'auteur sur une oeuvre peut céder ce droit, en totalité ou en partie, d'une façon générale, ou avec des restrictions territoriales, pour la durée complète ou partielle de la protection; il peut également concéder, par une licence, un intérêt quelconque dans ce droit; mais la cession ou la concession n'est valable que si elle est rédigée par écrit et signée par le titulaire du droit qui en fait l'objet, ou par son agent dûment autorisé.

Le titulaire peut être l'auteur, mais dans plusieurs cas, le titulaire est l'employeur de l'auteur:

13.(3) Lorsque l'auteur est employé par une autre personne en vertu d'un contrat de louage de service ou d'apprentissage, et que l'oeuvre est exécutée dans l'exercice de cet emploi, l'employeur est, à moins de stipulation contraire, le premier titulaire du droit d'auteur; mais lorsque l'oeuvre est un article ou un autre contribution, à un journal, à une revue ou à un périodique du même genre, l'auteur, en l'absence de convention contraire, est réputé posséder le droit d'interdire la publication de cette oeuvre ailleurs que dans un journal, une revue ou un périodique semblable.

La seule exception dans la cession du droit d'auteur est lorsque le document est associé au droit d'auteur de la Couronne, qui appartient au gouvernement du Canada .

12. Sous réserve de tous les droits ou privilèges de la Couronne, le droit d'auteur sur les oeuvres préparées ou publiées par l'entremise, sous la direction ou la surveillance de Sa Majesté ou d'un ministère du gouvernement, appartient, sauf stipulation conclue avec l'auteur, à Sa Majesté et, dans ce cas, il subsiste jusqu'à la fin de la cinquantième année suivant celle de la première publication de l'oeuvre.

Les gouvernements, en particulier le gouvernement fédéral, ne disposent pas d'un mécanisme simple de cession de droit d'auteur pour ce genre de biens de la Couronne. C'est pourquoi lorsqu'un auteur est un fonctionnaire et que, par conséquent, le droit d'auteur appartient à la Couronne, une simple permission d'usage est suffisante pour l'éditeur, pour autant que celle-ci ait été octroyée par une personne jouissant de l'autorité nécessaire pour ce faire.

J.-D. Dutil, M. Castonguay, D. Gilbert, and D. Gascon	**1818–1831**	Growth, condition, and environmental relationships in Atlantic cod (*Gadus morhua*) in the northern Gulf of St. Lawrence and implications for management strategies in the Northwest Atlantic
Caroline Marchand, Yvan Simard, and Yves Gratton	**1832–1848**	Concentration of capelin (*Mallotus villosus*) in tidal upwelling fronts at the head of the Laurentian Channel in the St. Lawrence estuary
Yoji Narimatsu and Hiroyuki Munehara	**1849–1855**	Spawn date dependent survival and growth in the early life stages of *Hypoptychus dybowskii* (Gasterosteiformes)
Cecily C.Y. Chang, James Langston, Mike Riggs, Donald H. Campbell, Steven R. Silva, and Carol Kendall	**1856–1864**	A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations
Eva Wahlström and Erika Westman	**1865–1872**	Planktivory by the predacious cladoceran *Bythotrephes longimanus*: effects on zooplankton size structure and abundance
S.E. Campana, G.A. Chouinard, J.M. Hanson, and A. Fréchet	**1873–1881**	Mixing and migration of overwintering Atlantic cod (*Gadus morhua*) stocks near the mouth of the Gulf of St. Lawrence
Beth Scott, Gudrun Marteinsdottir, and Peter Wright	**1882–1890**	Potential effects of maternal factors on spawning stock–recruitment relationships under varying fishing pressure
Teija Seppä, Nina Peuhkuri, Heikki Hirvonen, Anssi Laurila, Jorma Piironen, and Esa Ranta	**1891–1897**	Narrow size regime among individuals favors rapid growth in Arctic char (*Salvelinus alpinus*) juveniles
Randy Brown and Kenneth P. Severin	**1898–1903**	Elemental distribution within polymorphic inconnu (*Stenodus leucichthys*) otoliths is affected by crystal structure
M.R.J. Sheehy, R.C.A. Bannister, J.F. Wickins, and P.M.J. Shelton	**1904–1915**	New perspectives on the growth and longevity of the European lobster (*Homarus gammarus*)
François Landry, John R. Post, and Eric A. Parkinson	**1916–1928**	Spatial ontogeny of lentic age-0 rainbow trout, *Oncorhynchus mykiss*: whole-lake manipulations of population size structure
Akio Imai, Takehiko Fukushima, and Kazuo Matsushige	**1929–1937**	Effects of iron limitation and aquatic humic substances on the growth of *Microcystis aeruginosa*
Serge Parent and Antoine Morin	**1938–1948**	The role of copepod-dominated meiofauna in the mineralization of organic matter in a cold marine mesocosm
PERSPECTIVES		PERSPECTIVES
Barbara Neis, David C. Schneider, Lawrence Felt, Richard L. Haedrich, Johanne Fischer, and Jeffrey A. Hutchings	**1949–1963**	Fisheries assessment: what can be learned from interviewing resource users?
K. Cuddington and P.R. Leavitt	**1964–1977**	An individual-based model of pigment flux in lakes: implications for organic biogeochemistry and paleoecology
OBITUARY		NÉCROLOGIE
	1978–1980	Terrance Henry Butler

National Research Council Canada
Ottawa, Canada K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada K1A 0R6

Postage paid at Ottawa
Publications mail
Registration No. 1240579

Port payé à Ottawa
Poste-publication
Enregistrement n° 1240579

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 56, Number 10, October 1999

Volume 56, numéro 10, octobre 1999

RAPID COMMUNICATIONS — COMMUNICATIONS RAPIDES

M. Omair, Henry A. Vanderploeg, David J. Jude, and Gary L. Fahnenstiel **1711–1715** — First observations of tumor-like abnormalities (exophytic lesions) on Lake Michigan zooplankton

Brian J. Pyper and Randall M. Peterman **1716–1720** — Relationship among adult body length, abundance, and ocean temperature for British Columbia and Alaska sockeye salmon (*Oncorhynchus nerka*), 1967–1997

ARTICLES — ARTICLES

William G. Clark **1721–1731** — Effects of an erroneous natural mortality rate on a simple age-structured stock assessment

G.M. Fowler and W.T. Stobo **1732–1751** — Effects of release parameters on recovery rates of tagged groundfish species

Gregory T.O. LeBreton, F. William H. Beamish, and Robert G. Wallace **1752–1756** — Lake sturgeon (*Acipenser fulvescens*) growth chronologies

H. Perrein-Ettajani, J.C. Amiard, J. Haure et C. Renaud **1757–1765** — Effets des métaux (Ag, Cd, Cu) sur la composition biochimique et compartimentation de ces métaux chez deux microalgues *Skeletonema costatum* et *Tetraselmis suecica*

Marja-Liisa Koljonen, Håkan Jansson, Tiit Paaver, Oleg Vasin, and Jarmo Koskiniemi **1766–1780** — Phylogeographic lineages and differentiation pattern of Atlantic salmon (*Salmo salar*) in the Baltic Sea with management implications

Barb Crosbie and Patricia Chow-Fraser **1781–1791** — Percentage land use in the watershed determines the water and sediment quality of 22 marshes in the Great Lakes basin

Claire Vanier, Dolores Planas, and Michel Sylvestre **1792–1800** — Empirical relationships between polychlorinated biphenyls in sediments and submerged rooted macrophytes

Robert M. Holmes, Alain Aminot, Roger Kérouel, Bethanie A. Hooker, and Bruce J. Peterson **1801–1808** — A simple and precise method for measuring ammonium in marine and freshwater ecosystems

Rafael Morales-Baquero, Presentación Carrillo, Isabel Reche, and Pedro Sánchez-Castillo **1809–1817** — Nitrogen–phosphorus relationship in high mountain lakes: effects of the size of catchment basins

Continued on inside back cover / Suite au verso

Canada

Printed in Canada by / Imprimé au Canada par
University of Toronto Press Inc.
Typesetting by / Typographie par
NRC Research Press / Presses scientifiques du CNRC

0706-652X(199910)56:10;1-N